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(71) Applicant: BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS [US/US]; 506 South Wright Street, Urbana, IL 61801 (US).

(72) Inventors: KATZENELLENBOGEN, John, A.; 704 West Pennsylvania Avenue, Urbana, IL 61801 (US). KATZENELLENBOGEN, Benita, S.; 704 West Pennsylvania Avenue, Urbana, IL 61801 (US). FINK, Brian, E.; 526 Camino del Mar #36, Del Mar, CA 92014 (US). STAUFFER, Shaun, R.; 219 Nottingham, Springfield, IL 62704 (US). MORTENSEN, Deborah, S.; 502 West Main Street, Urbana, IL 61801 (US). SATTIGERI, Viswajanani, Jitendra; 2108 South Orchard Street #303, Urbana, IL 61801 (US). HUANG, Ying; Apartment 13, 1080 Baytowne Drive, Champaign, IL 61821 (US).

(74) Agents: SULLIVAN, Sally, A. et al.; Greenlee, Winner and Sullivan, P.C., Suite 201, 5370 Manhattan Circle, Boulder, CO 80303 (US).

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(54) Title: ESTROGEN RECEPTOR LIGANDS

(57) Abstract

This invention provides non–steroidal estrogen receptor ligands having a modular structure that is amenable to solid phase synthesis and the application of combinatorial synthetic methods to prepare these estrogen receptor ligands. ER ligands of this invention consist of a core scaffold that is a carbocyclic or heterocyclic–5–member ring that has two double bonds or a 6–member aromatic ring. A plurality of selected substituents are bonded to the ring substantially independently of other substituents. The modular structure of these compounds allows for synthesis of a very large numer of substituent structural variations, substituent combinations and substituent positioning on the core. The structural variants of the ER ligands of this invention exhibit a spectrum of selective affinities for ERα and ERβ and a spectrum of agonist/antagonist properties.

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ESTROGEN RECEPTOR LIGANDS

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application takes priority under 35 U.S.C § 119(e) from U.S. provisional application serial number 60/102,881 filed October 2, 1999 which is incorporated in its entirety by reference herein.

BACKGROUND OF THE INVENTION

Estrogens are endocrine regulators of the female reproductive system that also have important effects in many non-reproductive tissues (bone, liver, cardiovascular system, CNS, etc.). Many estrogen pharmaceuticals, based on both natural and synthetic substances, have been developed as agents for regulating fertility, preventing and controlling hormone-responsive breast cancer, and menopausal hormone replacement. These substances display a spectrum of agonist to antagonist activity that can show remarkable tissue and cell selectivity [Grese, T.A. et al. (1997), "Molecular determinants of tissue selectivity in estrogen receptor modulators," Proc. Natl. Acad. Sci. USA 94:14105-14110].

The molecular target of estrogens is the estrogen receptor (ER), of which there are now known to be two subtypes, ER-α and ER-β, that have different patterns of tissue expression and

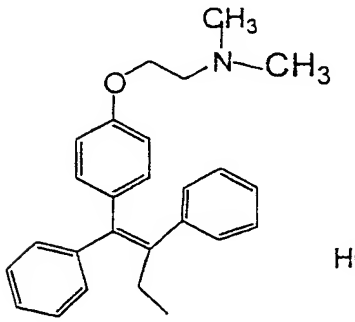
somewhat different ligand binding specificities [Mosselman, S. et al. (1996), "ERβ: Identification and characterization of a novel human estrogen receptor," FEBS Lett **392**:49-53; Kuiper, G.G. J.M. et al. (1996), "Cloning of a novel receptor expressed in rat prostate and ovary," Proc. Natl. Acad. Sci. USA **93**:5925-5930]. ER is a transcription factor that binds to specific estrogen response elements in the promoter region of estrogen-regulated genes and whose activity for transcription is modulated by the estrogen ligands [Katzenellenbogen, J.A. and Katzenellenbogen, B.S. (1996), "Nuclear hormone receptors: ligand-activated regulators of transcription and diverse cell responses," Chem. Biol. **3**:529-536]. The capacity of ER-ligand complexes to activate gene transcription is mediated by a series of co-regulator proteins [Horwitz, K.B. et al. (1996), "Nuclear receptor coactivators and corepressors," Mol. Endocrinol. **10**:1167-1177]. These co-regulators have interaction functions that tether ER to the RNA polymerase II preinitiation complex, as well as enzymatic activities to modify chromatin structure [Glass, C.K. et al. (1997), "Nuclear receptor coactivators," Curr. Opin. Cell. Biol. **9**:222-232]. Each cell type and each gene presents to an ER(subtype)-ligand complex a unique combination of these effector components – various estrogen response elements and co-regulators – that appear to underlie, in part, the cell and gene selectivity of various estrogens [Katzenellenbogen, J.A. et al. (1996), "Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones," Mol. Endocrinol. **10**:119-131]. Tissue specificity and differences in agonist/antagonist activity of ER ligands may also, at least in part, be attributed to differences in ligand activity with or affinity for different sub-types of the ER receptor.

One third of all breast carcinomas are hormone-responsive and nearly all of these are estrogen-positive [Henderson, I.C., Cannellos G.P. (1980) *New Eng. J. Med.* **320**:17]. For patients with estrogen-responsive tumors, hormonal therapies are preferred over cytotoxic chemotherapy and radiotherapy regimens because of their lower toxicity and the possibility that further remissions can be achieved with sequential use of multiple endocrine regimens [Royce, C. (1993) *Drugs of the Future* **18**:599-600].

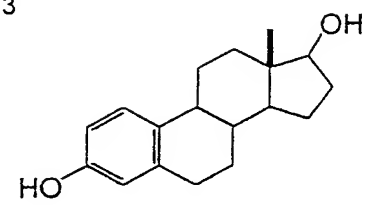
Among known ligands for ER, the natural estrogens are the simplest of the steroidal hormones, distinguished by having a phenolic A-ring. Synthetic estrogens, especially those of non-steroidal nature, generally retain a phenolic function (at least for those of high potency), but otherwise span a remarkable range of structural motifs that encompass simple acyclic core structures of various lengths and sizes, as well as a variety of ring-size fused and non-fused carbocyclic and heterocyclic systems [Magarian, R.A. et al. (1994), "The medicinal chemistry of nonsteroidal antiestrogens: A review," Curr. Med. Chem. 1:61-104; Solmssen, U.V. (1945), "Synthetic estrogens and the relation between their structure and their activity," Chem. Rev. 37:481-598]. Minor changes in the structure and stereochemistry of these ligands can have profound effects on both their affinity for ER, as well as their biocharacter (i.e., agonist vs antagonist balance). Major efforts have been directed toward optimizing ER ligand structure to obtain desired profiles of tissue selectivity, and even so, the ideal profile for various uses has not yet been achieved [Grese, T.A. et al. (1997), "Molecular determinants of tissue selectivity in estrogen receptor modulators," Proc. Natl. Acad. Sci. USA **94**:14105-14110; Grese, T.A. et al. (1998), "Synthesis and pharmacology of conformationally restricted raloxifene analogues: highly potent selective estrogen receptor modulators," J. Med. Chem. **41**:1271-1283].

Tamoxifen, the ER ligand most commonly employed in hormonal therapy for estrogen-positive breast cancer [Jordan, V.C. (1995) Breast Cancer Res. Treat. **36**:267-285], is a mixed agonist/antagonist for ER. This drug exhibits a number of side effects when used in breast cancer therapy. The level of agonist-antagonist activity of tamoxifen is variable and tissue dependent [Katzenellenbogen, B.S. (1996) *Biol.Repro*d. **54**:287-293 and Katzenellenbogen, J.A. et al. (1996) *Mol. Endocrinol.* **10** :119-131]. Tamoxifen may increase the incidence of liver and uterine cancer [Davidson, N. (1995) New Eng. J. Med. **332**.:1638-1639 and Katzenellenbogen, B.S. (1991) J. Natl. Cancer Inst.**83** :1434-1435]. In contrast, the stimulatory effects of tamoxifen in bone cells can be beneficial for the prevention of osteoporosis in postmenopausal women [Katzenellenbogen, B.S. (1996) *Biol.Repro*d. **54**:287-293]. Pure antiestrogens, such as ICI 164,384 also show promise for hormonal therapy for estrogen-positive breast cancer, but exhibit detrimental effects on other estrogen positive tissues (bone, central nervous system and

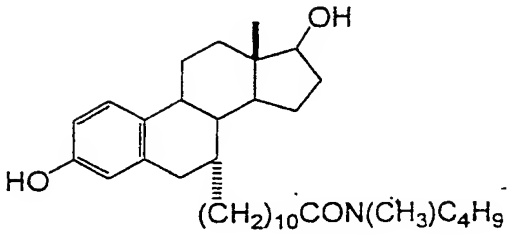
the cardiovascular system). A selective endocrine profile, as yet not achieved, which effects the



Tamoxifen



Estradiol



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desired inhibitory response in targeted tumor cells, while avoiding detrimental inhibitory or stimulatory effects in other tissues, is preferred in a drug for use in hormonal therapy for estrogen-positive breast cancer.

Combinatorial chemistry is of significant current interest for the identification of drug candidates. Combinatorial synthetic methods involve the parallel synthesis of a large collection of structurally related analogs to generate a library of compounds representing systematic structural variations that is then available for functional assessment. Combinatorial libraries are most often screened for a selected biological activity or function. Assessment of the properties of the members of such libraries of structurally related compounds can provide valuable insight into the relationship between structure and the property or function assessed. Combinatorial synthetic techniques have been applied extensively to the generation of large peptide libraries [Gallop, M.A. et al. (1994) "Applications of combinatorial technologies to drug discovery. 1. Background and peptide combinatorial libraries." J. Med. Chem. 37:1233-1251]. More recently, analogous techniques employing solid-phase organic synthesis have been applied to the development of non-peptide libraries [Bunin, B.A. and Ellman, J.A. (1992), "A general and expedient method for the solid-phase synthesis of 1,4-benzodiazepine derivatives," J. Am. Chem. Soc. 114:10997-10998; Bunin, B.A. et al. (1994), "The combinatorial synthesis and chemical and biological evaluation of a 1,4-benzodiazepine library," Proc. Natl. Acad. Sci. USA 91:4708-4712; Hobbs-

Dewitt, S. et al. (1993), "'Diversomers': an approach to nonpeptide, nonoligomeric chemical diversity," Proc. Natl. Acad. Sci. USA **90**:6909-6913; Beebe, X. et al. (1992), "Polymer-supported synthesis of 2,5-disubstituted tetrahydrofurans," J. Am. Chem. Soc. **114**:10061-10062; Chen, C. et al. (1994), "Analogous organic synthesis of small-compound libraries: validation of combinatorial chemistry in small-molecule synthesis," J. Am. Chem. Soc. **116**:2661-2662; and Zuckerman, R.N. et al. (1994), "Discovery of nanomolar ligands for 7-transmembrane G-protein coupled receptors from a diverse (N-substituted)glycine peptoid library," J. Med. Chem. 37:2678-2685]. Combinatorial techniques have been applied to the synthesis of pyrazoles from 1,3-diketones on solid support [Marzinzik, A.L. and Felder, E.R. (1996) Tetrahedron Lett. 37:1003] and to the synthesis of various heterocycles from α,β-unsaturated ketones [Marzinzik, A.L. and Felder, E.R.(1998) J. Org. Chem. 63:723-727]. Solid-state synthesis of tetra- and penta-substituted pyrroles has been reported [Mjalli A.M.M. et al. (1996) Tetrahedron Lett. 37:2943-2946].

For the most part, ER ligands currently under investigation are not well suited for synthesis by combinatorial approaches, because their preparation generally involves a series of carbon-carbon bond forming reactions that are not uniformly high yield, nor well adapted to solid phase synthetic methods. For example, combinatorial approaches using solid phase synthetic methods have been applied to the preparation of ER ligands having stilbene-like structures [Williard, R., et al. (1995) Curr. Biol. 2:45-51 and Brown, D.S. and Armstrong, R.W. (1996) J.Am. Chem. Soc. **118**:6331-6332]. However, combinatorial approaches have had limited application to the preparation of ER ligands.

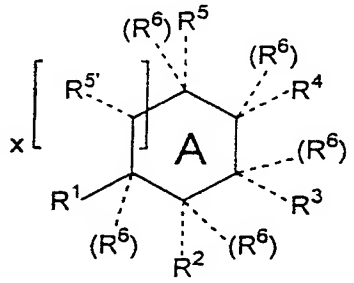
The present invention is based, at least in part, on the inventors' development of a simple modular pharmacophore for ER ligands consisting of a core structure linked to a plurality of independent substituents. The identification of this modular generic structure for ER ligands led to the development of modular stepwise synthetic methods, adaptable to solid-phase chemistry, for the generation of a combinatorial library of potential ER ligands with systematic structural

variation. Structural variants are readily generated based on this pharmacophore by variation of the core structure and selection of the substituents to be linked to the core structure.

SUMMARY OF THE INVENTION

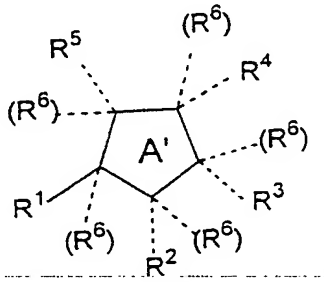
This invention provides non-steroidal estrogen receptor ligands having a modular structure that is amenable to solid phase synthesis and the application of combinatorial synthetic methods to prepare these estrogen receptor ligands. ER ligands of this invention consist of a core scaffold to which a plurality of selected substituents can be bonded substantially independently of other substituents. The modular structure of these compounds allows for synthesis of a very large number of substituent structural variations, substituent combinations and substituent positioning on the core. The structural variants produced by combinatorial methods can be assessed for differences in ER binding affinity and differences in physiological function allowing selection, for example, of ER ligands with a desired spectrum of agonist/antagonist properties. The ability to rapidly identify and select ER ligands with differences in agonist/antagonist properties allows the identification and selection of ER ligands optimized for a given clinical or pharmaceutical application.

The compounds of this invention consist of a core structure that carries up to 6 substituents which together provide for binding to and interaction with ER. The core scaffold is a 5 -membered ring structure that is doubly unsaturated (two double bonds) or 6-membered ring structure which is aromatic (triply unsaturated). The ring structure can be a carbocyclic ring or a heterocyclic ring have one or two non-carbon heteroatoms in the ring. The core ring can be described by the general formula:



where x is 0 or 1 and where the core ring A is a carbocyclic or heterocyclic ring having two double bonds, if it is a 5-membered ring (x = 0) or that is aromatic, if it is a 6-membered ring (x = 1). The positioning of double bonds and heteroatoms in the ring is not illustrated in the structure above, but various ring structures are illustrated in Tables 1 and 2. Substituents attached via dotted bonds are optional, dependent upon double bond and heteroatom placement. The substituents in parenthesis are potentially present in compounds that have 5-membered ring cores.

In one aspect, the core scaffold is a 5-membered doubly unsaturated ring structure which can be a carbocyclic ring, i.e. a cyclopentadiene, or a heterocyclic ring having one or two non-carbon elements, e.g., O, S or N, heteroatoms in the ring. ER ligands of this structure have the general formula:



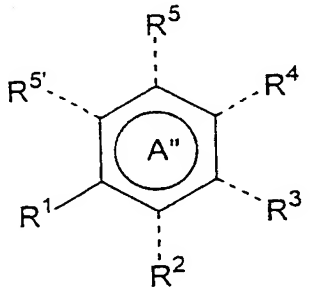
where the 5-membered ring A' can be a variety of carbocyclic and heterocyclic moieties with various positioning of two double bonds. Substituents attached with dotted lines are optional, dependent upon the position of double bonds and any heteroatoms in the ring. The possible double bonds of ring A' and the possible heteroatoms, which can be placed in various ring positions, are not shown in the structure above. A variety of core ring A' structures illustrating placement of double bonds, heteroatoms and substituents are exemplified in Table 1.

Table 1 contains a number of exemplary core 5-membered ring structures illustrating positioning of R substituents on the 5-membered ring. Core ring structures are exemplified by cyclopentadienes, cyclopentadienones, pyrazoles, imidazoles, oxazoles, thiazoles, isoxazoles, isothiazoles, furans, pyrroles, and thiophenes. Additional structures may be obtained by varying

the relative placement of substituents and double bonds. Two adjacent R substituents on the 5-membered ring can together form a cyclic structure.

Dependent upon the core structure, the position of the heteroatom(s) relative to a particular R group and/or the placement of double bonds, the core can accommodate up to 3 to 6 substituents. Substituent R^1 is required in the compounds of this invention. All 5-membered ring cores accommodate a minimum of 3 substituents (R^1 and two of R^2, R^3, R^4 and R^5). It is preferred that both R^1 and R^3 or R^1 and R^4 be present and that they are both non-hydrogen substituents. It is more preferred that R^1, R^2, and R^3 or R^1, R^2, and R^4 be present and that each is a non-hydrogen substituent. Certain cores may accommodate one (e.g., pyrroles) or two (e.g., cyclopentadienes) additional substituents, R^5 and R^6 (which can be hydrogens), on the ring atoms. Bonds to these substituents are indicated by dashed lines and each possible R^5 and R^6 is placed within parentheses to indicate that they are not present in all core structures and that the position of R^6 can be varied in certain cores. Typically there will only be one R^6 substituent in a given 5-membered ring core, which, however, may be at any ring position. In cyclopentadienones, CR^4R^6 or CR^5R^6 can represent C=O.

In a second aspect, the core scaffold is a 6-membered aromatic ring structure which can be a carbocyclic ring, i.e. a benzene, or a heterocyclic ring, e.g., a pyrazine or a pyrimidine, having one or two non-carbon elements, e.g., O, S or N, heteroatoms in the ring. ER ligands of this structure have the general formula:



where the 6-membered aromatic A" ring can be a variety of carbocyclic and heterocyclic moieties. The possible locations of heteroatoms in the ring are not shown in the above structure. One or two of the substituents attached by dotted lines can be absent dependent upon the placement of heteroatoms. A variety of core ring structures illustrating placement of heteroatoms and substituents in six-membered rings are exemplified in Table 2. Two adjacent R substituents on a 6-membered core ring can together form a cyclic structure, as illustrated by quinoxalines or quinazolines in Table 2.

Dependent upon the core structure, the position of the heteroatom(s) relative to a particular R group and/or the placement of double bonds, the aromatic core can accommodate 4 to 6 substituents. Substituent R^1 is required in the compounds of this invention. Six-membered ring cores accommodate a minimum of 4 substituents (R^1 and three of R^2, R^3, R^4, R^5 and $R^{5'}$). Substituent $R^{5'}$ is selected from the same groups as R^5 and may be the same or different from R^5. R^3 or R^1 and R^4 be present and that they are both non-hydrogen substituents. It is more preferred that R^1, R^2, and R^3 or R^1, R^2, and R^4 be present and that each is a non-hydrogen substituent. Benzenes have six substituents. Pyridines have five substituents. Pyrimidines and pyrazines have four substituents. Bonds to substituents other than R^1 are indicated by dashed lines to show that they may be absent dependent upon heteroatom placement.

Substituent R^1 can be selected from the group consisting of phenyls and substituted phenyls wherein the non-hydrogen phenyl group substituents can include, without limitation, halogens (F, Cl, and Br, being preferred), hydroxy groups, lower alkyl, alkenyl, alkynyl, and alkoxyl groups (where the term "lower" indicates 1 to about 6 carbon atoms), lower ethers, ketones, or thioethers, and substituted lower alkyl, alkenyl or alkynyl groups (where the substituents can be halogens or hydroxy groups). Substituted alkyl, alkenyl and alkynyl groups can include perhalogenated groups, e.g., CF_3 or CF_2CF_3. The R^1 phenyl ring can carry multiple substituents that can be the same or different. Phenyl ring substituents can be at any of the ortho- (o-), meta- (m-) or para- (p-) positions on the ring. Preferred substituents of R^1 phenyl groups are halogens (particularly F, Cl and Br), methyl, ethyl, vinyl, methoxy, ethoxy and hydroxy groups.

Preferred substituted phenyl groups are para-substituted, particularly p-halogen- and p-hydroxy-substituted phenyls. The most preferred R^1 group is p-hydroxyphenyl. In general, the R^1 phenyl group can carry any substituent that can be metabolically converted into a p-OH group, e.g., OCH_3, $O\text{-}COCH_3$, etc.

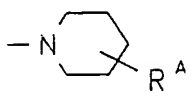
Substituents R^2, R^3 and R^4, can be the same or different, and can be selected from the group consisting of hydrogen, a phenyl or substituted phenyl group (where phenyl substitution is as described for R^1), lower alkyl, alkenyl or alkynyl (where the lower alkyl, alkenyl or alkynyl groups may be substituted, with a phenyl, hydroxyls or halogens), lower ethers, ketones or thioethers, and halogens (F, Cl, Br, or I). Where a halogen is directly attached to the core ring, Br and I are preferred halogens. Substituted alkyl, alkenyl and alkynyl groups can include perhalogenated groups, e.g., perfluorinated groups, such as CF_3 or CF_2CF_3. Substituted alkyl, alkenyl and alkynyl groups include those substituted with a phenyl ring or a substituted phenyl ring, e.g., benzyl, p-hydroxybenzyl, m-fluorobenzyl, etc. Preferred R^2, R^3, R^4 are lower alkyl or alkenyl groups, and phenyl and o-, m-, or p-substituted phenyl groups. More preferred R^2 are ethyl and propyl (straight-chain or branched) groups. More preferred R^4 are phenyls, o-, — or p-hydroxyphenyl, o-, m-, or p-alkoxyphenyl, o-, m-, or p-halophenyl, and branched alkyl groups, e.g., a t-butyl group. More preferred R^3 are phenyls and substituted phenyls, including o-, m-, or p-hydroxyphenyl, o-, m-, or p-alkoxyphenyl, o-, m-, or p-halophenyl.

Substituents R^5 or $R^{5'}$ when present, e.g., in pyrroles and cyclopentadienes and other 5-member ring systems (Table 1) or aromatic systems (Table 2), can be selected from any of the groups defined for R^2, R^3 and R^4 and may be hydrogens. R^5 and $R^{5'}$ may be the same as or different than any of R^1, R^2, R^3, or R^4. Substituents R^5 or $R^{5'}$ may be the same or different than each other.

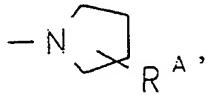
R^6 when present, e.g. in cyclopentadienes, can be hydrogen, lower alkyl, alkenyl, alkynyl, or alkoxy groups, substituted lower alkyl, alkenyl or alkynyl groups, lower ether or thioethers or a halogen (particularly F, Cl and Br). Substitution of alkyl, alkenyl and alkynyl R^6 groups can

include halogen and hydroxy group substitution. One or more of any $-CH_2-$ groups in R^6 can be replaced with -CO- groups. R^6 is preferably a lower alkyl or hydrogen.

In specific embodiments, the R^1-R^4 (and R^5, $R^{5'}$ or R^6 when present) can also carry or be basic or polar groups, e.g., an alkyl, phenyl or other substituent listed above that carries a basic or polar substituent or basic or polar groups directly linked to the core ring structure. Basic and/or polar groups on ER ligands can provide the ligand with antagonist or mixed agonist/antagonist properties. Basic groups include without limitation: amines, and amine-substituted alkyl, alkenyl or alkoxy groups. Amines can be alkyl, alicyclic or aromatic amines. Basic groups specifically include: $-(X)_x(CH_2)_n-NRR'$ where X is O or S, x is 0 or 1, n is an integer from 1 to about 10 and preferably 2 to 6, and R and R', can be the same or different and can be alkyl, aryl, or alicyclic. R and R' in these specified basic groups can together form an heterocyclic or a substituted heterocyclic ring, e.g.,



or



where R^A (which may represent multiple substituents) can be lower alkyl, alkenyl or alkynyl groups or substituted lower alkyl, alkenyl or alkynyl groups. Additionally, any alicyclic rings can contain one or more carbonyl groups -CO-.

In general any polar groups can be employed as substituents on any R groups or for direct linkage to the ring. Preferred polar groups include halogens, perhalogenated alkyl, alkenyl or alkynyl groups, hydroxy groups, hydroxy-substituted alkyl, ethers or thioethers, diols, amides, sulfoxides, and sulfones, e.g.,:

Diols: $-(X)_x(CH_2)_n-CH(OH)-CH(OH)-R^B$ where X is O or S, x is 0 or 1, n is an integer from 1 to about 6 and preferably 1 to about 4, R^B is H or $-(CH_2)_m-CH_3$, where m is an integer from 0 to about 6. Diols can also be alicyclic;

Amides:

$-(X)_x(CH_2)_nCO-NRR'$ where X is O or S, x is 0 or 1, n is an integer from 1 to about 12 and preferably 6 to about 10, and R and R', can be the same or different and can be alkyl, aryl, alicyclic, substituted alkyl, substituted aryl or substituted alicyclic, or together form an heterocyclic or a substituted heterocyclic ring; and

Sulfoxides:

$-(X)_x(CH_2)_nSOR'$ where X is O or S, x is 0 or 1, n is an integer from 1 to about 12, including those with n = 1 to about 5 and those with n= 6 to about 10, and R' can be alkyl, aryl, alicyclic, substituted alkyl, substituted aryl or a substituted alicyclic ring where substituents include halogens, particularly F, Cl and Br and perhalogenated alkyl groups, such as CF_3 and CF_2CF_3;

Sulfones:

$-(X)_x(CH_2)_nSO_2R'$ where X is O or S, x is 0 or 1, n is an integer from 1 to about 12, including those with n = 1 to about 5 and those with n= 6 to about 10, and R' can be alkyl, aryl, alicyclic, substituted alkyl, substituted aryl or a substituted alicyclic ring where substituents include halogens, particularly F, Cl and Br and perhalogenated alkyl group, such as CF_3 and CF_2CF_3.

See Scheme 17 for exemplary basic and polar substituents.

Each of the hydroxy-substituted alkyls and the above-listed amides, diols, sulfoxides and sulfones can be directly attached to the 5-membered core ring as an R^2-R^6 or $R^{5'}$ substituent or can be a substituent on any of R^1-R^6 or $R^{5'}$.

In five-membered ring compounds any two substituents on a given ring carbon can be linked to form a spiro-ring. For example, substituents R^5 and R^6 or R^4 and R^6 on the same ring atom can together form a carbon chain $-(CH_2)_n-$ where n is 3 to about 6 to form a spiro ring

system with the parent cycle A. Carbons in the R^5/R^6 chain or R^4/R^6 chain may also be substituted, e.g., with halogens or lower alkyl, alkenyl or alkynyl groups, and one or two of the CH_2 groups of the chain may be replaced with an -CO-, -O- , -S- or an -NH-.

Substituents on adjacent ring atoms, e.g., the pairs R^1 and R^2, R^2 and R^3, R^3 and R^4, R^1 and R^5, $R^{5'}$ or R^6, can be linked to form a saturated or unsaturated carbocyclic or heterocyclic ring structure fused to the parent cycle A, e.g., an alkyl substituent of R^2 can be linked to a phenyl substituent at R^1 or R^3.

Substituents are generally selected independently of core ring size, as discussed above to achieve desired ER ligand characteristics, but are preferably also selected to provide stable compounds and facilitate ease of preparation.

In specific embodiments, 5- and 6-member ring ER ligands of this invention can contian two substituted or non-substituted phenyl rings in addition to R^1. In other specific embodiments, R^1 is substituted at a ring atom directly adjacent to a ring atom substituted with a lower alkyl group, particularly an ethyl, n-propyl, i-propyl, i-butyl or n-butyl group. In other specific embodiments, R^1 is a p-substituted phenyl group, where the substituent is OH or OR where R is a lower alkyl group, R^2 is a lower alkyl group (up to C6) and the ligand contains in addition one or two substituted or non-substituted phenyl groups. Preferred substituents on the additional phenyl rings are p-OH, m-OH, p-halogen, m-halogen, p-OR or m-OR where R is a lower alkyl group.

In more preferred embodiments, the ER ligands of this invention have core structures as listed in Tables 1 and 2 where the substituents R^1-R^6 and $R^{5'}$ are as defined above. Structural variants in addition to those listed in Table 1 and 2 may be obtained by interchanging the positions of R^2, R^3, R^4, and R^5. ER ligands are those compounds which exhibit measurable binding affinity for the estrogen receptor in assays as described herein.

Exemplary compounds of this invention are provided in Scheme 18.

. The non-steroidal ER ligands of this invention are useful in pharmaceutical compositions for the treatment of hormone-responsive disorders. The non-steroidal ER ligands of this invention are particularly useful in pharmaceutical applications for treatment of estrogen-responsive disorders and conditions, as active ingredients of pharmaceutical compositions in combination with a pharmaceutically acceptable carrier or exipient. The ER ligands may be combined with each other to achieve a desired pharmaceutical response or administered in combination with known estrogens or antiestrogens. The ER ligand is present in the pharmaceutical compositions in an amount, or in combination with other ligands in a combined amount, sufficient to induce or inhibit estrogen response. In those cases in which the ER ligand selectively interacts with an ER subtype or variant, the amount of ligand (or combined amount of ligands) present in the pharmaceutical composition is in the range that induces or inhibits the desired selective response. The invention also relates to methods of treating estrogen responsive disorders and physiological conditions employing pharmaceutical compositions comprising ER ligands of this invention alone or in combination. This invention provides pharmaceutical compositions which comprise one or a mixture of ER ligands having structures disclosed herein in combination with a pharmaceutically acceptable carrier appropriate for the pharmaceutical application and compatible with the ER ligand. ER ligands are present in these pharmaceutical compositions in an amount or in a combined amount sufficient to elicit a measurable positive effect on a symptom or condition associated with an estrogen-dependent disorder on administration to an individual suffering from the symptom or disorder.

Pharmaceutical compositions of this invention can also include other steroid or non-steroid ER ligands which may supplement or enhance the activity of the composition for a particular medical application. Pharmaceutical compositions of this invention include those which are useful in the prevention and treatment of hormone-dependent cancers, including breast cancer, those useful for hormone-replacement therapy, those useful in the treatment of infertility, those useful for treatment of osteoporosis and those useful for providing cardiovascular, CNS (suppress hot flashes, provide cognitive improvements, etc.) or related benefits. Pharmaceutical compositions of this invention can be provided in a variety of dosage

forms including without limitation pills for oral administration, solutions or emulsions for oral administration or for injection.

This invention also provides methods for the treatment of hormone-dependent disorders, including the treatment of hormone-responsive breast cancer, which comprise the step of administering to a patient having the disorder or symptoms thereof a pharmaceutical composition comprising one or a mixture of the ER ligands of this invention where the ER ligand or mixture of ligands is present in the composition at a level or a combined level sufficient to effect a positive biological response.

ER ligands of this invention can exhibit agonist, antagonist or mixed agonist/antagonist function in vitro and in vivo. These functions can be assessed for a given ER ligand or ligand mixture employing in vitro methods known in the art or as described in the Examples herein. This invention also provides methods for generation of and testing of combinatorial libraries of potential ER ligands for ER binding affinity as well as for the assessment of agonist/antagonist character of a given ER ligand.

The ER ligands of this invention are useful in vitro and/or in vivo for selective activation or repression of expression, dependent upon the agonist or antagonist nature of the ligand, of a gene regulated by ER. Gene activation or repression can be selective with respect to subtype of ER (e.g., ERα or ERβ), or variant of ER (e.g., splice variant forms, truncated or processed forms, covalently modified forms, etc.).

The ER ligands of this invention are also useful in vitro and/or in vivo for selective regulation of cellular activities under the control of ER. Cellular activities may be regulated in a variety of ways by ER, subtypes of ER or variants of ER, e.g., up or down regulation of a given cellular process. Regulation is selective with respect to subtype of ER (e.g., ERα or ERβ), or variant of ER (e.g., splice variant forms, truncated or processed forms, covalently modified forms, etc.). Cellular activities that may be regulated include both genomic (related to gene

expression) or non-genomic activities (not directly related to gene expression, e.g., such as regulation of calcium flux, particularly in bone cells, hormone release, particularly prolactin release from pituitary cells, etc.).

The subtype-selective ER ligands of this invention can also be of general use in the investigation of ER and its functions. These ligands can be employed to better understand structure and conformation of ER (both subtypes) and to elucidate how ER subtypes interact with other molecules and to relate structure, conformation and interaction with other molecules to ER function.

BRIEF DESCRIPTION OF THE DRAWINGS

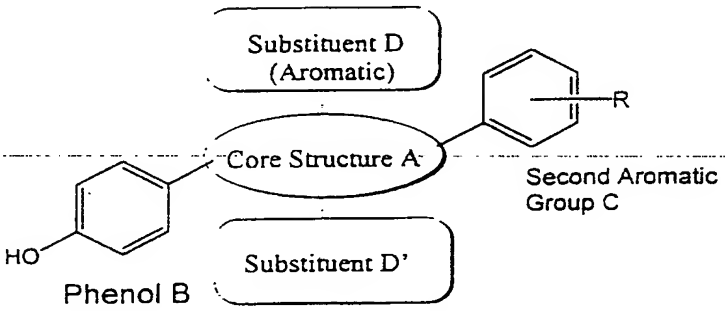
Figures 1A and 1B illustrate transcriptional activation by ERα and ERβ, respectively, in response to the pyrazole compound **38b**. Human endometrial cancer (HEC-1) cells were transfected with expression vectors for ERα (Fig. 1A) or ERβ (Fig. 1B) and an (ERE)3-pS2-CAT reporter gene and were treated with the indicated concentrations of estradiol (E2) or the pyrazole for 24 h. Cat activity was normalized for β-galactosidase activity from an internal control plasmid. Values are the mean ± SD for three or more separate experiments, and are expressed as a percent of the ERα and ERβ response with 10 nM E2. See: J. Sun et al. (1999) Endocrinology **140** (2):800-804.

Figure 2 illustrates transcriptional activation by ERα and ERβ in response to two pyrazoles XXX (solid lines) and XXX1(dashed lines). HEC-1 cells were transfected with expression vectors for ERα (diamonds) and ERβ (squares) and an $(ERE)_3$-pS2-CAT reporter gene and were treated with indicated concentrations of ligand for 24 h. CAT activity was normalized for β-galactosidase activity from an internal control plasmid. Values are the mean ± SD for three or more separate experiments, and are expressed as a percent of the ERα and ERβ response with 1 nM E2.

.. Figures 3A and 3B are transcriptional activation profiles for ERα (Fig. 3A) and ERβ (Fig. 3B) in response to pyrazole XXXX. HEC-1 cells were transfected with expression vectors for ERα or ERβ and an $(ERE)_3$-pS2-CAT reporter gene and were treated with indicated concentrations of ligand for 24 h in the presence or absence of estradiol (1 nM). CAT activity was normalized for β-galactosidase activity from an internal control plasmid. Values are the mean ± SD for three or more separate experiments.

DETAILED DESCRIPTION OF THE INVENTION

The pharmacophore model for ER ligands of this invention consists of a core structure onto which independent peripheral structural elements are attached. A preferred pharmacophore is illustrated in which a phenolic unit (B) that is always preserved, a second aromatic group (C) that is usually present, and another substituent (D) or two (D'), one of which may be aromatic is attached to the core (A):

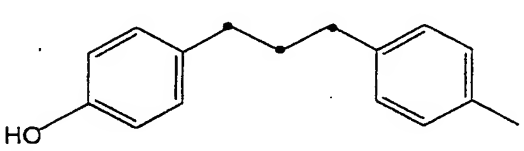


High ER binding affinity will be associated with certain geometric arrangements of the peripheral substituents (B-D'), so that they will be "in register" with their corresponding subsites in the ligand binding pocket in ER. Peripheral group orientation can be accomplished by core elements that encompass some structural variety. The core serves as a molecular scaffold whose function is to correctly orient the peripheral substituents with appropriate topology for high

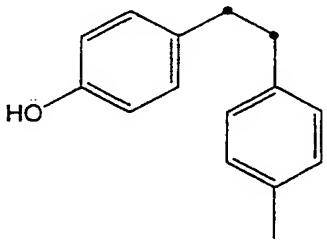
affinity ER binding. Further, the chemical nature of the core may effect the binding affinity and/or influence the interaction of substituents with ER.

In part because they allow some flexibility in orientation of substituent groups and in part because their synthesis is amenable to solid-phase methods, five-member and six-member carbocyclic and heterocyclic rings were selected for preparation of ER ligands based on the illustrated pharmacophore.

Two substructural motifs noted in known ER ligands are the homobibenzyl motif A, exemplified in the known non-steroidal ligands benzestrol and raloxifene and the syn-bibenzyl motif B. The A motif can be represented in various 3,5-diaryl-1,2-azoles (pyrazoles and isoxazoles) and various 2,4-diaryl-1,3-azoles (imidazoles, thiazoles, and oxazoles). The B motif can be represented in various 4,5-diaryl-1,3-azoles, as well as various 3,4-diaryl-1,2-azoles and various 4,5-diaryl-1,2-azoles. The structure of ER ligands of this invention expand from these basic motifs.



Motif A. Homobibenzyl

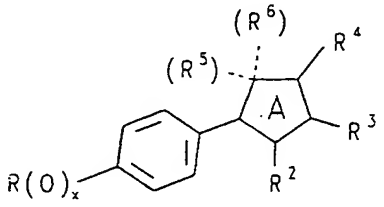


Motif B. Syn-Bibenzyl

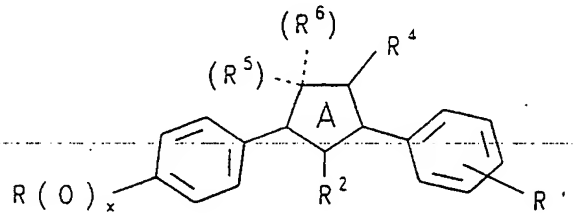
Tables 1 and 2 illustrate representative core five- and six-membered ring structures of the ER ligands of this invention. The cores include five-member cyclic rings that are doubly unsaturated and which may contain one or two heteroatoms (particularly N, O or S) . The cores also include six-member aromatic rings which may contain one or two heteroatoms (particularly N). The selected cores can accommodate from three to six substituents which can be oriented by placement on ring elements. The representative cores listed are distinct from one another in the

position of the R^1 substituent on the selected rings with respect to heteroatoms (and/or double bonds) and other substituents therein, in the cyclopentadienes with respect to the tetrahedral carbon, or in the cyclopentadienones with respect to the C=O group on the ring. Dependent upon the selection of a particular R^1-R^6 or $R^{5'}$ substituent distinct structures may be obtained by interchange of substituents.

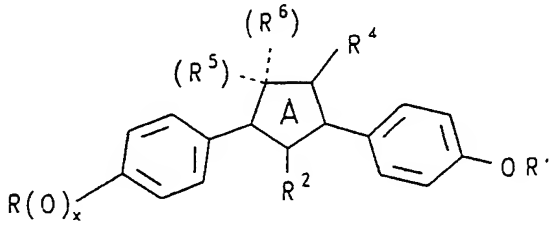
In specific embodiments, ER ligands of this invention can have the structures:



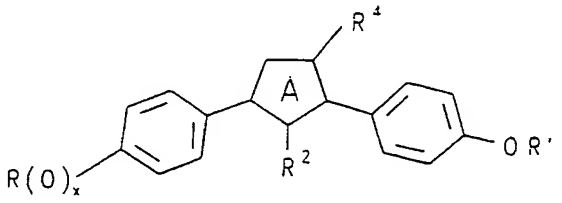
where x is 0 or 1 and R is hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other substituents are defined as in the Summary above;



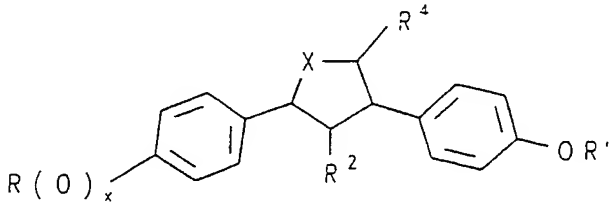
where x is 0 or 1 and R is hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, where R' is a phenyl ring substituent as defined above in the definition of R^3 and can be a polar or basic substituent and other variables are defined as in the Summary above;



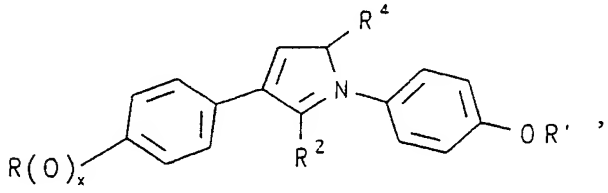
where x is 0 or 1, R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;



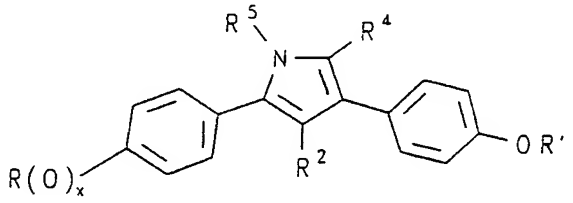
where x is 0 or 1, R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;

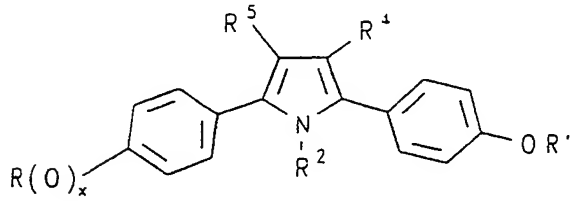


where x is 0 or 1, X is N, NH, NR^5, S or O and R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;

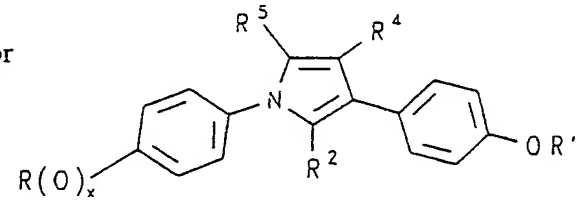


,

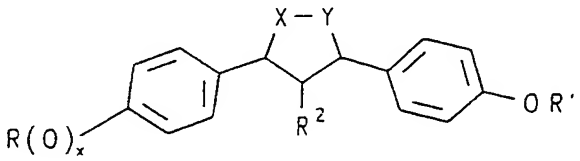




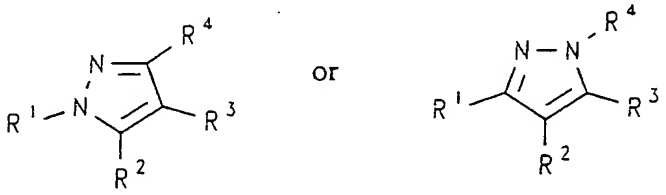
or



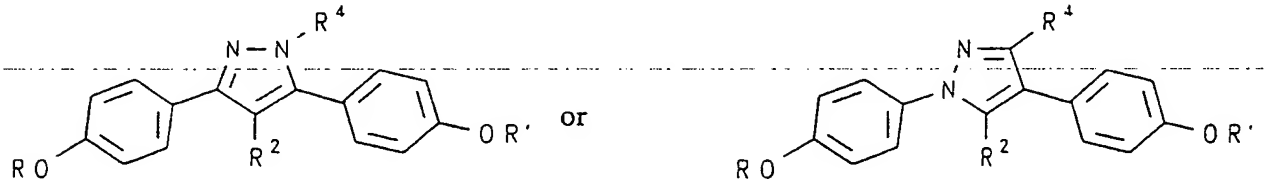
where x is 0 or 1, X is N, NH, NR^5, S or O and R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;



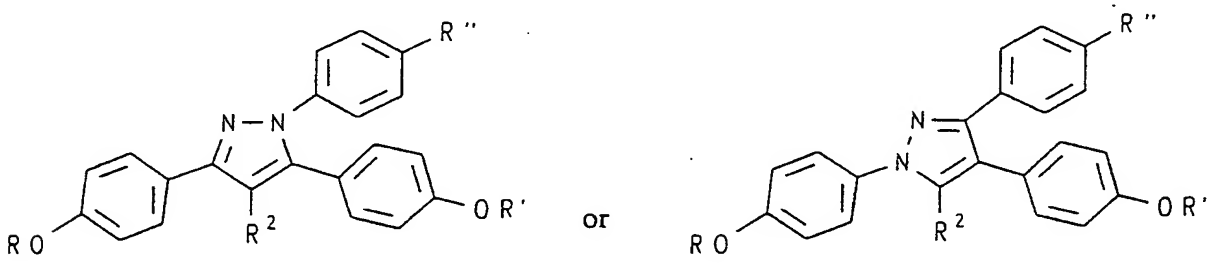
where one of X or Y is N and the other of X or Y is N, S or O, x is 0 or 1 and R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;



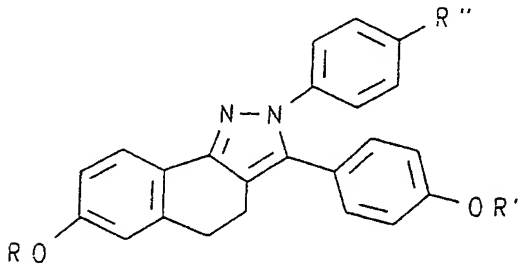
where R^1-R^4 are defined as in the Summary above;



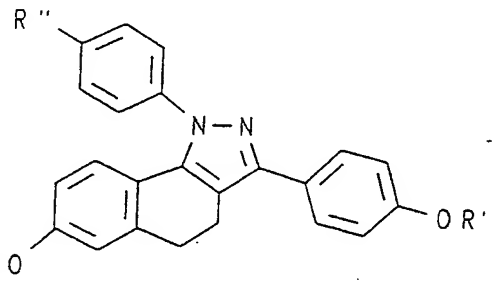
where R^2 and R^4 are defined as in the Summary above and R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group;



where R^2 is defined as in the Summary above, R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and R" can be a hydrogen, a halogen, a hydroxy, an alkoxy, or a basic or polar group; and



or

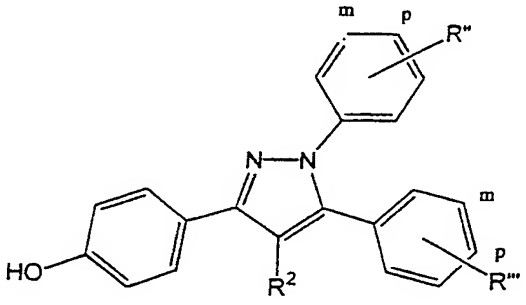


where R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and R" can be a hydrogen, a halogen, a hydroxy, an alkoxy, or a basic or polar group.

Preferred R and R' are H, preferred R" is OH and preferred R^2 are straight-chain or branched lower alkyl groups having up to 6 carbons atoms.

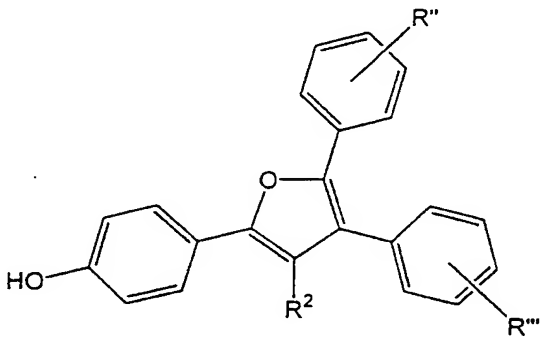
Specific compounds having the illustrated structures are listed in Scheme 18.

Pyrazoles of particular interest having significant ER binding affinity (RBAs) include those having the structure:



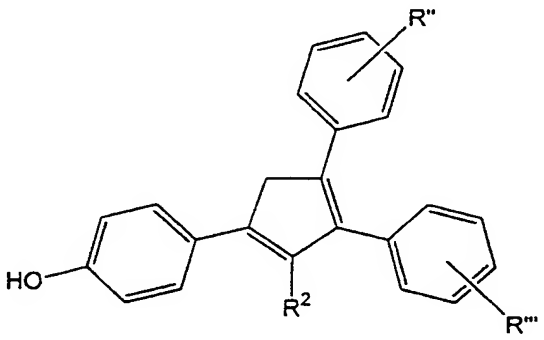
where R^2 is an ethyl, n-propyl, isopropyl, n-butyl, isobutyl or t-butyl group, R″ and R‴ may be positioned at the *meta* or *para* ring positions and can be selected independently of each other from the group p-H, p-OH, p-F, p-Br, p-CH_3, m-OH, m-F, or m-Br. Pyrazoles of this structure exhibiting generally higher ER affinity are those in which R‴ and R″ are both p-OH.

Furans of particular interest having significant ER binding affinity (RBAs) include those having the structure:



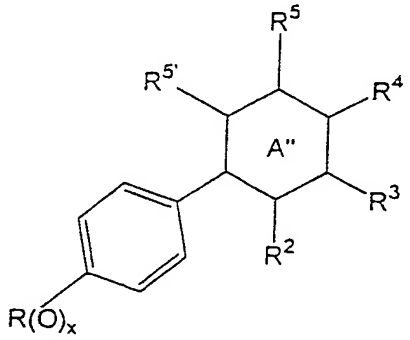
where R^2 is an ethyl, n-propyl, isopropyl, n-butyl, isobutyl or t-butyl group, R″ and R‴ may be positioned at the *meta* or *para* ring positions and can be selected independently of each other from the group p-H, p-OH, p-F, p-Br, p-CH_3, m-OH, m-F, or m-Br. Furans of this structure exhibiting generally higher ER affinity are those in which R‴ and R″ are both p-OH.

Cyclopentadienes of particular interest include those having the structure:

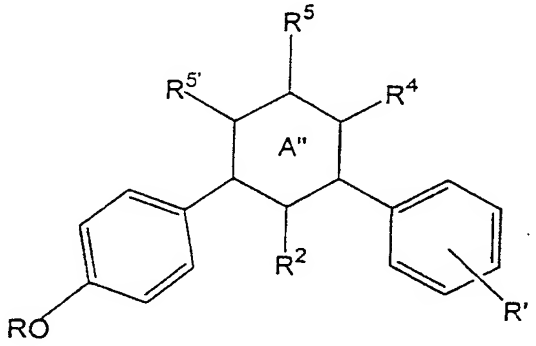


where R^2 is an ethyl, n-propyl, isopropyl, n-butyl, isobutyl or t-butyl group, R″ and R‴ may be positioned at the *meta* or *para* ring positions and can be selected independently of each other from the group p-H, p-OH, p-F, p-Br, p-CH_3, m-OH, m-F, or m-Br. Furans of this structure exhibiting generally higher ER affinity are those in which R‴ and R″ are both p-OH.

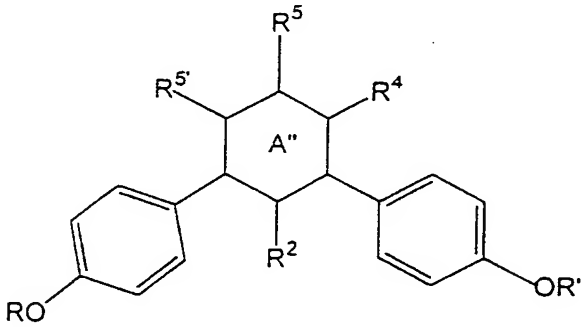
In specific embodiments, ER ligands of this invention can have the structures:



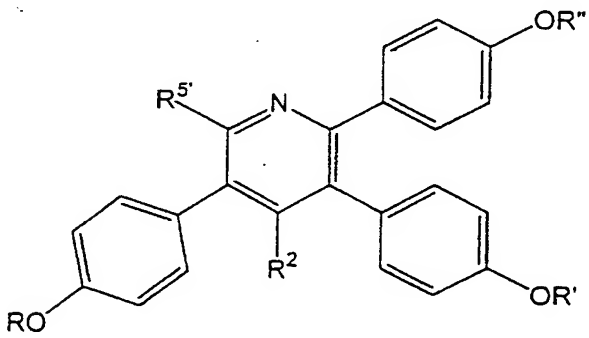
where the A″ is an aromatic ring with up to two heteroatoms in the ring, x is 0 or 1 and R is hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, other substituents are defined as in the Summary above, and one or two of the indicated substituents may be absent due to heteroatom placement;

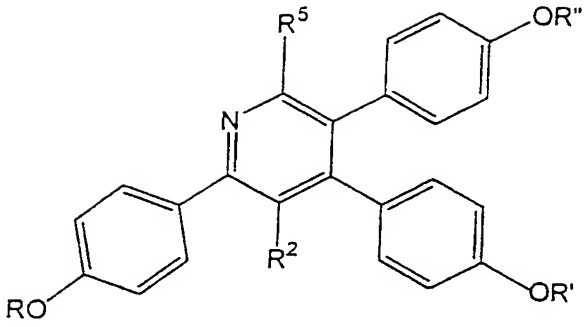


where A" is an aromatic ring with up to two heteroatoms in the ring, R is hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, R' is a phenyl ring substituent as defined above in the definition of R^3 which may be at any ring position (preferably para or meta ring positions) and can be a polar or basic substituent, other substituents are defined as in the Summary above, and one or two of the indicated substituents may be absent due to heteroatom placement;

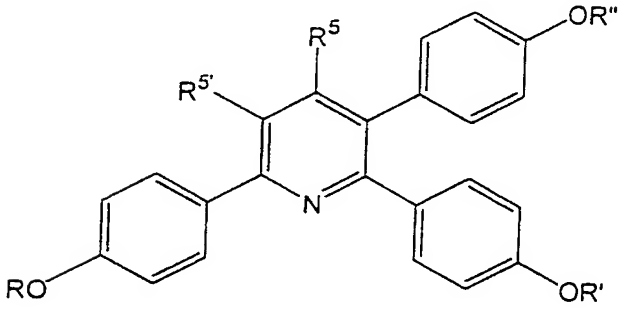


where R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, other variables are defined as in the Summary above and one or two of the indicated substituents may be absent due to heteroatom placement;





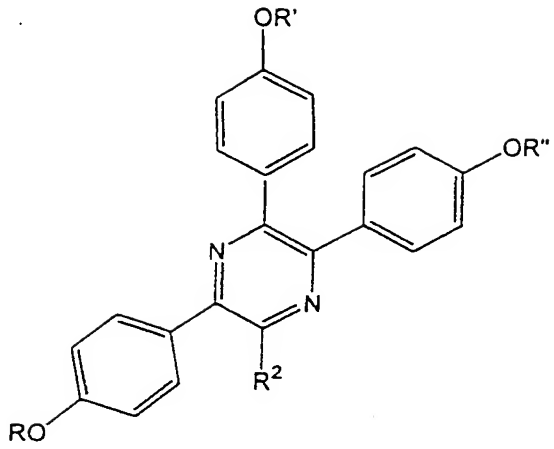
or

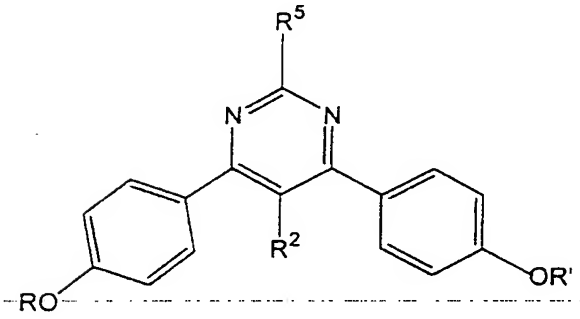


where R, R' and R" can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;

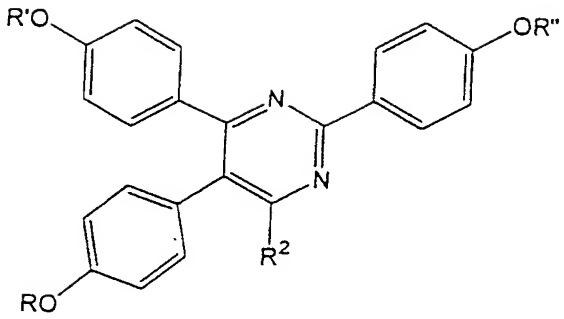
5

or



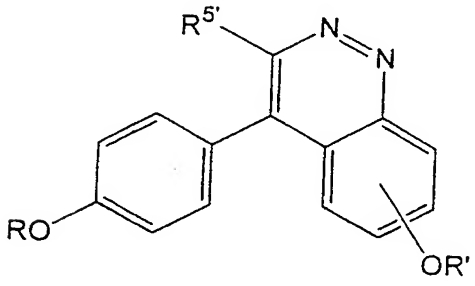


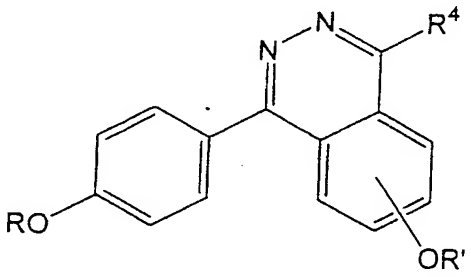
where R, R' and R" can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, other variables are defined as in the Summary above;

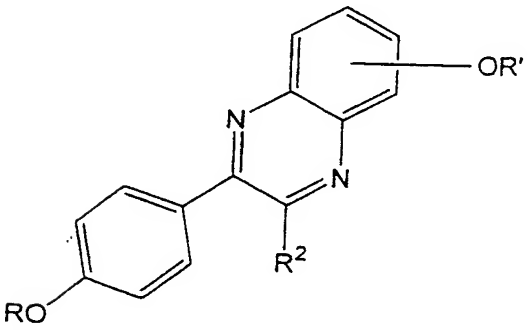


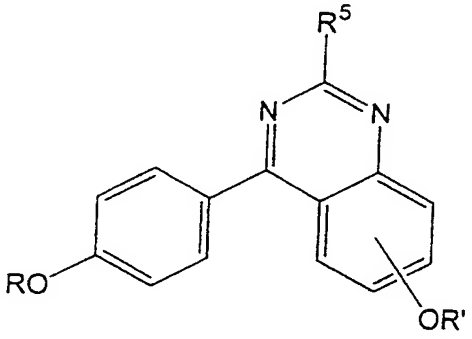
where R, R' and R"can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group where R^2 is defined as in the Summary above, but is preferably a lower alkyl groups having up to about 6 carbon atoms; and

Any of:

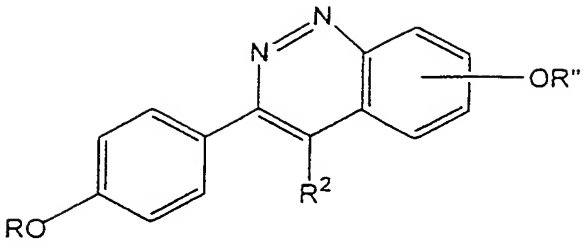






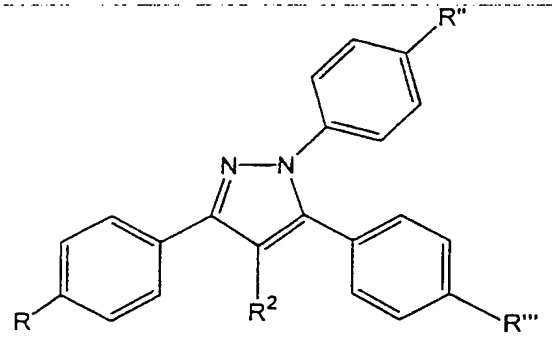


or



where R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, other substituents are as described above in the Summary .

Five- and six-membered ring core compounds of this invention substituted with a basic or polar side chain are of particularly interest as potential SEEMS (selective estrogen receptor modifiers) which can display agonist or antagonist activity (or a mixture of these activities) that can vary from tissue to tissue. Basic or polar side chain can be substituted at several possible positions in the compounds of this invention. For example, in pyrazoles of the following structure:



a basic or polar group can be substituted at R, R^2, R"or R'". A preferred positioning of the basic or polar side chain is such that it would occupy a region of the ligand binding pocket normally occupied by such groups in known SERMS, as well as complete estrogens antagonists. Basic side groups of particular interest for substitution into pyrazoles include those carrying alicyclic

amine groups, e.g.,: $-(X)_x(CH_2)_n-NRR'$ where X is O or S, x is 0 or 1, n is an integer from 1 to about 10 and preferably 2 to 6, and R and R', can be the same or different and can be alkyl, aryl, or alicyclic. R and R' in these specified basic groups can together form an heterocyclic or a substituted heterocyclic ring. A preferred basic side group is a piperidinylethoxy group. Preferred R^2 are lower alkyl groups having up to about 6 carbon atoms.

Synthesis of ER Ligands

Imidazoles – The synthesis of representative symmetrical members of the imidazole class (specifically core structure IM1 of Table 1) and their N-alkyl analogs is accomplished by a well known approach [Sarshar, S., Siev, D. & Mjalli, A.M.M. (1996). Imidazole libraries on solid support. *Tetrahedron Lett.* **37**, 835-838] as shown in Scheme 1A. Refluxing 4,4'-dimethoxybenzil (**1**) in formamide in the presence of *para*-formaldehyde affords the 4,5-disubstituted imidazole **2** [Bredereck, H., Gompper, R. & Hayer, D. (1959). Imidazole aus α-Diketonen. *Chem. Ber.* **92**, 338-343], which upon deprotection with BBr_3 in CH_2Cl_2 affords the imidazole **3** in good yield. A similar reaction using 4-methoxybenzaldehyde affords the 2,4,5-trisubstituted imidazole **4** [Lombardino, J.G. & Weisman, E.H. (1974), "Preparation and antiinflammatory activity of some nonacidic trisubstituted imidazoles,". *J. Med. Chem.* 17, 1182-1188; Schubert, V.H., Giesemann, G., Steffen, P. & Bleichert, J. (1962), "p-Aryl- und p-Alkoxyphenyl-imidazole," *J. Prakt. Chem.* **18**, 192-202; Hayes, J.F., Mitchell, M.B. & Wicks, C. (1994), "A novel synthesis of 2,4,5-triarylimidazoles," *Heterocycles* **38**, 575-585]. To prepare tetrasubstituted systems, the sodium salt of imidazole **4** is alkylated with ethyl, propyl, and butyl iodide, and then deprotected to afford free phenols **6a-d**.

Unsymmetrical, imidazoles (IM1 core) are synthesized as outlined in Schemes 1B and 2. Scheme 1B illustrates the synthetic approach to N-ethyl imidazole **12**. Reaction of 4-methoxy-deoxybenzoin (**7**) [Gardner, P.D. (1956), "Organic peracid oxidation of some enol esters involving rearrangement," *J. Am. Chem. Soc.* **78**, 3421-3424] with bromine and a trace of $AlCl_3$ in Et_2O gives α-bromoketone **8** [Jenkins, S.S. (1934) "The grignard reaction in the synthesis of

ketones. IV. A new method of preparing isomeric unsymmetrical benzoins,". *J. Amer. Chem. Soc.* 56, 682-684] which, upon reaction with sodium azide in acetone, affords the corresponding azide **9**. The azido-ketone **9** is treated with one equivalent of Et_3N and imine **10** in THF. Removal of solvent and excess Et_3N followed by treatment of the crude intermediate 2,5-dihydro-2-hydroxyimidazole with TFA in CH_2Cl_2, according to the procedure of Patonay [Patonay, T. & Hoffman, R.V. (1995), "Base-Promoted Reactions of α-Azido Ketones with Aldehydes and Ketones: A Novel Entry to α-Azido-β-hydroxy Ketones and 2,5-Dihydro-5-hydroxyoxazoles," *Journal of Organic Chemistry* **60**, 2368-2377], results in the formation of *N*-ethyl imidazole **11**. Deprotection with $BF_3 \cdot SMe_2$ in CH_2Cl_2 produces imidazole **12** in good yield.

The N-aryl substituted imidazoles (IM2 core of Table 1), as exemplified by imidazole **17** can be synthesized as outlined in Scheme 2. Refluxing 4'-methoxy-α-bromobutyrophenone (**13**) with *p*-anisidine in acetone gives the α-amino-ketone **14**, which is converted into the benzamide **15** upon reaction with benzoyl chloride and base. Cyclization with ammonium acetate in refluxing acetic acid affords the 1,2,4,5 tetrasubstituted imidazole **16**, which upon deprotection with $BF_3 \cdot SMe_2$ in CH_2Cl_2 produces the free phenol **17**.

The methods illustrated in Schemes 1A, 1B and 2 can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having imidazole ring core structures. Details of syntheses of representative imidazole are provided in the Examples.

Thiazoles – The synthesis of representative thiazoles is shown in Scheme 3. Thioamide **19** (when R' = Me), derived from 4-alkoxybenzonitrile (**18** when R' = Me) [Taylor, E.C. & Zoltewicz, J.A. (1960), "A new synthesis of aliphatic and aromatic thioamides from nitriles," *J. Am. Chem. Soc.* **82**, 2656-2657] is condensed with 4'-alkoxy-α-bromoacetophenone (**20** when R' = Me) or 4'-alkoxy-α-bromobutyrophenone (**13** when R' = Me) in refluxing DMF to give the 2,4-disubstituted thiazole, e.g., **21a**, [Dolling, K., Zaschke, H. & Schubert, H. (1979), "Kristallin-

flussige Thiazole," *J. Prakt. Chem.* 321, 643-654] or 2,4,5-trisubstituted thiazole, e.g., **21b**, respectively. Deprotection with BBr_3 affords moderate yields of the free phenols, e.g., **22a** and **22b**. Details of a representative syntheses are provided in the Examples.

The methods illustrated in Scheme 3 can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having thiazole ring core structures.

Oxazoles – Representative oxazoles can be synthesized, as outlined in Schemes 4A and B. Reaction of the lithium anion of dithiane **23** with *p*-methoxybenzyl bromide gives the alkylated product (**24** when R = Me) which upon hydrolysis affords 4'-alkoxy-deoxybenzoin (**25** when R = Me) [Katrizky, A.R., Boulton, A.J. & Short, D.J. (1960), "Interaction at a distance in conjugated systems. Part III. Effect of aryl and heteroaryl groups on the infrared intensities of C=C and C+C stretching bands," *J. Chem. Soc.* , 1519-1523] in excellent yield. Conversion to the bromide (**26** when R = Me) [Cowper, R.M. & Stevens, T.S. (1940), "Mechanism of the reaction between arylamines and benzoins," *J. Chem. Soc.* , 347-349] and azide (**27** when R = Me) is accomplished as described for analogous compounds **8** and **9** above. The azido-ketone **27** is then treated with one equivalent of Et_3N and *p*-anisaldehyde, and then with TFA to afford oxazole (**28** when R = Me) [Strzybny, P.P.E., van ES, T. & Backeberg, O.G. (1969), "Reaction of α-acyloxyketones with ammonium acetate," *J. South African Chem. Inst.* 22, 158-164]. Oxazole **30** results from the condensation of bromo-ketone **26** with *p*-methoxybenzamide in refluxing toluene (Scheme 4B) analogous to the thiazole synthesis discussed above. Deprotection of **28** and **30** with $BF_3{\cdot}SMe_2$ gives oxazoles **29** and **31**, respectively. Details of a representative synthesis are provided in the Examples.

The methods illustrated in Schemes 4A and B can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having oxazole ring core structures.

Pyrazoles – The synthesis of the pyrazoles is illustrated in Schemes 5A-C. Scheme 5A involves the condensation of a hydrazine with a 1,3-diketone [Marzinzik, A.L. & Felder, E.R. (1996), "Solid support synthesis of highly functionalized pyrazoles and isoxazoles; scaffolds for molecular diversity," *Tetrahedron Lett.* **37**, 1003-1006]. The method of Beak [Reitz, D.B., Beak, P., Farney, R.F. & Helmick, L.S. (1978), "Dipole-stabilized carbanions from thioesters. Evidence for stabilization by the carbonyl group," *J. Am. Chem. Soc.* **100**, 5428-5436] can be used to obtain 1,3-diketone **33** from the reaction of the methyl thioester **32** and lithium tetramethylpiperidide. Condensation of the diketone with hydrazine hydrochloride or N-substituted hydrazine hydrochlorides in refluxing DMF/THF (1:1) affords the 3,5-disubstituted pyrazole **34a** or 1,3,5-trisubstituted pyrazoles **34b-d**; yields can be higher with aryl-substituted hydrazines than with hydrazine itself [van Steenis, J. (1946), "The nitration of dianisoylmethane and p-methoxydesoxybenzoin," *Chem. Ber.* 29-46; Hergenrother, P.M. (1991), "New Developments in Thermally Stable Polymers," *Rec. Travl. Chim. Pays-Bas.* **110**, 481-491; Ando, W., Sato, R., Yamashita, M., Akasaka, T. & Miyazaki, H. (1983), "Quenching of singlet oxygen by 1,3,5-triaryl-2-pyraazolines," *J. Org. Chem.* **48**, 542-546]. Deprotection of **34a-d** with BBr_3 affords the free phenols **35a-d**.

The introduction of a 4-alkyl substituent was accomplished through the alkylation of diketone **33** with TBAF and an alkyl iodide (e.g., ethyl iodide) to afford **36**. [Tewari, S.C. & Rastogi, S.N. (1979), "Studies in antifertility agents: Part XXII: 1,2-diethyl-1,3-bis-(p-hydroxyphenyl)-1-propene," *Ind. J. Chem.* **18B**, 62-64; Clark, J.H. & Miller, J.M. (1977), "Hydrogen bonding in organic synthesis. Part 6. C-Alkylation of β-dicarbonyl compounds using tetralkylammonium fluorides," *J. Chem. Soc., Perkin I*, 1743-1745] . Conversion of diketone **36** to the corresponding pyrazoles is accomplished as with the unsubstituted case, to afford pyrazoles **38a-d**. Details of representative syntheses are provided in the Examples.

Alternatively, ketone **90** can be reacted with 2 eq. of nitrobenzyl ester **91** and $LiN(iPr)_2$ to give the 1,3-diketone which is then taken to the pyrazole (e.g., **38**) as further indicated in Scheme 5A (Path B).

Scheme 5B provides more detail of the syntheses of pyrazoles **200-204** via the method of Scheme 5A.

Scheme 5C presents a general method for synthesis of pyrazoles having core PA2 in which R^1 is attached to a ring. This scheme also illustrates a method for addition of I to the ring. Any halogen can be added by appropriate selection of reagent. Scheme 5D provides more detail of the syntheses of pyrazoles **205-209** via the method of Scheme 5C.

The methods illustrated in Schemes 5A-D can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having pyrazole ring core structures.

Isoxazoles – An illustrative preparation of an isoxazole is shown in Scheme 6 [Perkins, M., Beam, C.F., Dyer, M.C.D. & Hauser, C.R. (1988), "3-(4-Chlorophenyl)-5-(4-methoxyphenyl)isoxazole," *Org. Syn. Coll. Vol.* **VI**, 278-281]. Double deprotonation of the ketoxime (**39** when R^2 is H) derived from 4-methoxyacetophenone with n-BuLi, followed by addition of methyl 4-methoxybenzoate affords the 3,5-disubstituted isoxazole e.g., **40** [Ichinose, N., Mizuno, K., Tami, T. & Otsuji, Y. (1988), "A novel NO insertion into cyclopropane ring by use of $NOBF_4$. Formation of 2-isoxazolines," *Chem. Lett.* , 233-236]. Deprotection with BBr_3 afforded the free phenol **41** [Murthy, A.K., Rao, K.S.R.K.M. & Rao, N.V.S. (1968) "Isoxazolylphenols and their absorption spectra," *Aus. J. Chem.* **21**, 2315-2317]. Details of a representative synthesis are provided in the Examples.

The methods illustrated in Scheme 6 can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having isoxazole ring core structures.

Isothiazoles- Illustrative preparations of isothiazoles are shown in Schemes 7A and B. Reaction of the thioketone imine **42** with iodine results in cyclization to form isothiazole **43**

(Scheme 7A). Alternatively, isoxazoles (as prepared in Scheme 6) can be reductively cleaved to form enaminoketone **44** which on treatment with P_2S_5 /chloranil results in isothiazole **43**.

The methods illustrated in Schemes 7A and B can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having isothiazole ring core structures.

Furans, Thiophens and Pyrroles: Heterocycles having one heteroatom in the 5-membered ring core (e.g., furans, thiophenes and pyrroles) can generally be prepared by cyclization of appropriately substituted 1,4-diketones. Ring substitution is for the most part determined by selection of the 1,4-diketone. The synthesis of 1,4-diketones is illustrated in Scheme 8. Starting with aldehydes and ketones that are commercially available or readily synthesized by well-known methods, substituted α, β-unsaturated ketones are formed by treatment with ethanolic KOH. The α, β-unsaturated ketones are transformed using, for example, the Stetter reaction with appropriately substituted aldehydes in the presence of a thiazolium salt catalyst (e.g., 3-benzyl-5-(2-hydroxyethyl)-4-methyl-thiazolium chloride for aliphatic aldehydes or 3-ethyl-5-(2-hydroxyethyl)-4-methyl-thiazolium bromide for aromatic aldehydes) to form the desired diketones. See: Khanna, I.K. et al. (1997) J. Med. Chem. **40** :1619-1633 and Stetter, H. (1976) Angewandte Chemie Int'l Ed. Eng. **15**: 639-647. Several exemplary diketones **53-56** are listed in Scheme 8. These diketones can be converted into furans (Scheme 8), thiophenes (Scheme 9) or pyrroles (Schemes 10A-D).

Furans- Acid catalyzed cyclization of the diketones, illustrated in Scheme 8 for diketones **53-56** give furans **57-59** in 85-93% yields (Wu, A. et al. (1997) Synthetic Comm. **27**:2087-2091). Also illustrated is addition of a halogen substituent (Y) to the furan ring. The synthesis of additional exemplary furans **210-214** is illustrated in Scheme 8A where a synthesis of 1,4-diketone precursors is illustrated at the top of the scheme.

An alternate approach to 1,4-diones is illustrated in Scheme 8B which uses enolate chemistry employing α-bromoketones as electrophiles. Desoxyanisions were treated with one equivalent of potassium bis(trimethylsilyl) amide followed by addition of α-bromoketone to give the desired tetra-substituted diones in good yield. This approach affords the 1,4-diones as mixtures of diastereomers, however, no separation of the stereoisomers is required, as these centers become non-stereogenic in the final products.

Thiophenes-Treatment of diketones as illustrated in Scheme 9 for diketones **54** and **56** with Lawesson's Reagent gives thiophenes **60** and **61** in about 85% yields [Kiebooms, R.H.L. et al. (1997) J. Org. Chem. **62**:1473-1480]. Also illustrated is addition of a halogen substituent (Y) to the furan ring.

Pyrroles- Acid catalyzed cyclization of diketones in the presence of a selected primary amine as illustrated in Schemes 10A-B and 10D for diketones (such as **53** and **55**) results in the formation of N-substituted pyrroles PR3 (e.g., **62** where $R^1 = R^3 = C_6H_4\text{-}OCH_3$, $R^2 = C_2H_5$ and $R^4 = C_6H_5$) and PR2 [Khanna, I.K. et al. (1997) J. Med. Chem. **40**:1619-1633]. Reaction of the a 1,4-diketone, e.g., **54**, with ammonium acetate in acetic acid results in a pyrrole PR2 where R^2 is H (such as **64** where $R^1 = R^3 = C_6H_4\text{-}OCH_3$, and $R^4 = C_6H_5$) in Scheme 10C. Deprotonation of the N-H pyrrole with sodium hydride followed by alkyl iodide addition (illustrated for ethyl iodide) gives the N-R^2-substituted analog . Scheme 10C also illustrates a method for introducing a halogen onto the pyrrole ring.

Scheme 10D illustrates a synthesis of a pyrrole of core structure PR1 (with N-R^1). It is apparent from an overview of Schemes 10A-D that a variety of different pyrroles with different relative positions of substituents R^1-R^5 with respect to each other and with respect to the N in the ring can be obtained by appropriate substitution of starting 1,4-diketones.

Furans, thiophene and pyrroles having methoxy substituents on substituted phenyl R groups (e.g. **58, 60** and **62**) can be deprotected with boron tribromide to afford the demethylated products.

The methods illustrated in Schemes 8, 8A, 9, and 10A-D can be employed or readily adapted by one of ordinary skill in the art using well-known techniques and methods for synthesis and with appropriate choice of starting materials for the preparation of the furans, thiophenes and pyrroles of this invention.

Cyclopentadienes and Cyclopentadienones: Scheme 11A (including paths A, B, B' and C) illustrates representative syntheses of cyclopentadienes and cyclopentadienones of this invention. Dieneone **93** produced for example by path A is cyclized to give the cyclic unsaturated ketone **94**. Additional non-hydrogen substituents e.g., R^4 and R^6, can be added to the five-membered ring as indicated in path C to ultimately give various cyclopentadienes (e.g., **95A-B**). The cyclic ketone **94** can be reduced via path B to a cyclopentadienone **96A**. Also, a cyclopentadiene having two hydrogens **97** on the same ring carbon can be oxidized to give a cyclopentadiene **96B**.

Scheme 11B illustrates a synthesis of cyclopentadienones of this invention and an alternative synthesis of cyclopentadienes. A cyclic unsaturated ketone **98** is prepared by cobalt carbonyl catalyzed cyclization of a substituted alkyne and olefin. This reaction can result in the generation of regioisomers. Ketone **98** is reduced to give cyclopentadiene **97**. Scheme 11C provides another general scheme for synthesis of cyclopentadienones.

Scheme 11D illustrates another alternative synthesis of cyclopentadienes of this invention as applied to cyclopentadiene ligands **230-235** with $R^2 = C_2H_5$. Cyclopentadienyl ligands with R^2 that is a lower alkyl group, e.g., n-propyl, can also be made by this method by selection of Grignard reagent. Compounds **236** and **237** where R^2 is n-propyl can also be made by this method. In this method, a thiazolium salt catalysed addition of an aldehyde to an α, β-

unsaturated ketone under Stetter conditions gives the corresponding 1,4-diketone. Intramolecular aldol condensation of the 1,4-diketone using methanolic potassium hydroxide gives cyclopentenones. Cyclopentadienes are derived from Gringard and dehydration reaction on the cyclopentenones. However, the cyclopentadienes were not stable to the conditions of deprotection to release free phenol. So the cyclopentenones were deprotected under mild conditiond (borontrifluoride-dimethylsulfide) to give the cyclopentenones. The free phenols were then temporarily reprotected as their trimethylsilyl ehters using bis(trimethysilyl)acetamide which were then subjected to Gringnard reaction. Dehydration of the tertiary alcohol and removal of the trimethylsilyl group were achieved under acidic work-up conditions following the Grignard reaction to give desired cyclopentadienes. The cyclopentadienes obtained were found to be very sensitive to air, heat and acidic impurities in solvents.

The methods illustrated in Schemes 11A-D can be employed or readily adapted by one of ordinary skill in the art using well-known techniques and methods for synthesis and with appropriate choice of starting materials for the preparation of the cyclopentadienes and cyclopentadienones of this invention.

Base Substituents: Schemes 12A-D illustrate representative methods for introduction of basic amino substituents into five-membered ring ER ligands of this invention. The schemes illustrate the synthesis of a base-substituted pyrazole. Intermediate **100** is reacted with a substituted 1,3 diketone to form the pyrazole. Schemes 12B and 12C illustrate in more detail the synthesis of Scheme 12A for the introduction of a piperidinylalkoxy basic group. Scheme 12B illustrates the introduction of the basic side chain at a ring nitrogen of a pyrazole. Scheme 12C illustrates the introduction of the basic side chain at C(3) of the pyrazole ring. Scheme 12 D illustrates introduction of a basic side chain on a phenyl substituent on the pyrazole ring.

The methods illustrated in Schemes 12A-D can be employed or readily adapted by one of ordinary skill in the art using well-known techniques and methods for synthesis and with

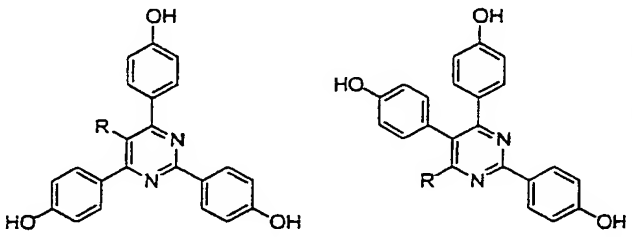
appropriate choice of starting materials for the preparation of various amine-substituted ER ligands of this invention.

Six-membered ring heterocycles

Pyridazine- six-membered ring pyridazine analogues can also be prepared from the 1,4-diones described above for synthesis of furans, thiophenes and pyrroles. As illustated in Scheme 13A, treatment of the diones with hydrazine hydrate followed by air oxidation affords the desired pyridazines. Exemplary pyridazines synthesized by the illustrated methods are indicated in Scheme 13A.

The method illustrated in Scheme 13 A can be employed or readily adapted by one of ordinary skill in the art using well-known techniques and methods for synthesis and with appropriate choice of starting materials for the preparation of the various substituted pyridazines ER ligands.

Pyrimidines- the pyrimidines are classified into two groups (see below) for convenience of description of their synthesis. The first group or Class-I compounds can be prepared as depicted in Scheme 13B. The following numbering applies to Scheme 13B. The reaction of ketone **1** with triflic anhydride in the presence of a nitrile **2** proceeds via a (trifluoromethanesulfonyl)carbenium ion to furnish the pyrimidines 3 in good yields. Deprotection of the phenolic methyl ether in 3 under mild acid conditions leads to the formation of the desired phenol-bearing pyrimidines 4 in good yields.



Pyrimidines : Class-I **Class-II**

For the synthesis of Class-II compounds of pyrimidines the synthetic sequence illustrated in Scheme 13C is employed. The following numbering applies to Scheme 13C. However, the use of a ketone 5, flanked by two methylene groups resulted in the formation of two separable regio-isomeric pyrimidines, obtained by the ring closure at either of the methylene carbons. Deprotection of the phenolic methyl ethers following the same strategy as in Scheme 13A lead to the formation of the regio-isomeric pyrimidines **6** and 7, respectively. The reaction, when extended to synthesize the pyrimidine **8** (with three phenolic groups), led to the formation of mostly the regio-isomer **9** with only trace amounts of the desired isomer **8**, which in addition was difficult to separate from the excess anisonitrile used in the reaction. Another synthetic procedure that can be applied to the synthesis the desired pyrimidine **8** is illustrated in Scheme 13D. This method can generally be applied to the synthesis of various pyrimidines of this invention.

The methods illustrated in Schemes 13A-D can be employed or readily adapted by one of ordinary skill in the art using well-known techniques and methods for synthesis and with appropriate choice of starting materials for the preparation of a variety of Pyrimidine ER ligands of this invention.

Pyrazines-the synthesis of the pyrazines follows a simple strategy as depicted in Scheme 13E. The following numbering refers to Scheme 13E. Condensation of diketone **10** with the substituted ethylenediamine **11** under acidic conditions furnished the pyrazines **13** in moderate yields. Deprotection of the phenolic methyl ethers of **13** under mild acid conditions furnished in good to excellent yields the pyrazines **13**. By another route, based on the condensation of α–hydroxy ketones with ammonium acetate in ethanol, as depicted in Scheme 13F, a mixture of the pyrazines were synthesized, which on deprotection under similar conditions as above gave

the pyrimidines 13, 14 (Scheme 13F) and an unseparable mixture of the pyrimidines 15 and 16(Scheme 13F).

Quinoxalines-the synthesis of the quinoxalines follows a simple strategy as depicted in Scheme14A. The following numbering refers to Scheme 14A. Condensation of the phenylenediamine 1 with α-diketone 2 under acidic conditions furnished in moderate yields a 1:1 mixture of the regio-isomeric quinoxalines 3. Removal of the phenolic methyl ether using boron triflouride dimethyl sulfide then furnished the deprotected quinoxalines 4 in good yields.

Quinazolines:-the synthesis of the quinazolines can be carried out using the strategy described in Scheme 14B.

Cinnolines-the synthesis of cinnolines can be carried out using the strategy described in Scheme 14C.

Phthalazines: the synthesis of phthalazines can be carried out using the strategy described in Scheme 14D.

Combinatorial Methods

Combinatorial chemistry can be employed to synthesize a variety of potential ER ligands having the 5-member and 6-membered unsaturated ring core structures described herein. These solid phase methods allow the production of a combinatorial library of compounds, having varying substituents on the core structure, to test for ER binding and activity. Schemes 15 A and B provides illustrative solid support syntheses of compounds having a heterocyclic ring structure, pyrazoles. These schemes exemplify the use of a resin P, e.g., the Merrifield resin, to tether a starting material. The synthesis proceed on the resin-tethered species and after formation of the desired substituted ring structure, it is released from the resin (i.e., solid support).

Scheme 15A illustrates distinct syntheses for compounds where R^3 is aliphatic (path A) or aryl (path B). This scheme can be used to generate pyrazoles with three or four substituents. Scheme 15B illustrates an alternate route to pyrazoles proceeding through a distinct intermediate **119** to a tethered pyrazole **109**. Path A in Scheme 15B illustrates halogen addition to the ring, e.g., **123**. The choice of paths in B depends on whether substituent R^2 is aliphatic or aryl.

Scheme 16 provides illustrative solid support syntheses of compounds having a heterocyclic ring structure, oxazoles, thiazoles and imidazoles. Interestingly, a single intermediate **134** in Scheme 16 can be used to generate compounds of all three ring structures **145**, **147**, or **149**.

Estrogen Receptor Binding

ER ligands are those compounds which exhibit measurable binding affinity for the estrogen receptor. There are various ways to measure and quantify ER binding affinity. In this invention ER binding affinity is measured in competitive binding assays compared to estradiol. Binding affinity is expressed as a relative binding affinity (RBA) in percent compared to estradiol which is assigned an affinity of 100%. Substantial affinity for ER is indicated by an RBA of about 0.1% or more. Good affinity binding to ER is indicated by an RBA of about 1%- to about 10%. High affinity binding to ER is indicated by an RBA of about 10% or higher.

The binding affinities of substituted compounds of heterocyclic cores structures listed in Table 1 are shown in Tables 3–5A-B, organized according to heterocyclic core structure. The binding values were obtained from a competitive radiometric binding assay, using [^{3}H]estradiol as the tracer and dextran-coated charcoal to adsorb free tracer or hydroxyapatite to adsorb the ER-tracer complex; the values are expressed as relative binding affinities (RBA), in percent, with respect to estradiol assuming an affinity of 100% for estradiol. Lamb and/or rat uterine cytosol ER preparations were used as described in Katzenellenbogen, J.A. et al. (1977) "Estrogen photoaffinity labels. 1. Chemical and radiochemical synthesis of hexestrol diazoketone and azide derivatives; photochemical studies in solution." Biochemistry **16**:1964-1970.

In some cases, a mixture of regioisomers were prepared and the binding affinity of the mixture was assessed. In particular in the unsymmetrical pyrazole cases (R1 ≠ R3), isomers were formed. In the cases so far studied, the isomers have been found to be formed in comparable amounts, so that the ratios of isomers in the mixtures are likely to be between 2:1 and 1:2. In some cases, the mixtures have been separated and the individual isomers have been tested for binding as pure compounds and significant differences in binding affinity have been found. In most cases, it has not yet been determined which regioisomeric structure corresponds to which separated regioisomer.

If the isomer ratios are within 1:2 or 2:1, then any binding affinity measured for a mixture could never be less than one-third the affinity of the pure high affinity isomer. In the worst case, if one isomer were inactive, and the other active isomer were present as the 1 part in a 1:2 mixture, then when the high binding isomer was pure, it concentration in the binding assay would be 3-fold higher and its measured affinity also 3-fold higher than in the 1:2 mixture. This means that in cases where mixtures have been examined for binding affinity, that one of the isomers present may have up to a 3-fold higher binding affinity than indicated by the measurement.

Without wishing to be bound thereby, the following analysis of the ER binding affinities of individual compounds is provided:

Imidzoles, Oxazoles and Thiazoles – The receptor binding data for several imidazoles are shown in Table 3. Although the members of this series have rather low affinity, there is an increase in RBA with the addition of alkyl substituents at the 1-position (**6a-d**); this trend reaches a maximum for propyl **6c**, reversing for the butyl substituent **6d**. Such trends are well known both in steroidal systems (11β- and 16α-substituents) [Anstead, G.M., Carlson, K.E. & Katzenellenbogen, J.A. (1997). The estradiol pharmacophore: ligand structure-estrogen receptor binding affinity relationships and a model for the receptor binding site. *Steroids* **62**, 268-303], as well as in other non-steroidal ligand series (such as 2-phenylindoles [von Angerer, E., Prekajac, J. & Strohmeier, J. (1984). 2-Phenylindoles. Relationship between structure, estrogen receptor

affinity, and mammary tumor inhibiting activity in the rat. *J. Med. Chem.* 27, 1439-1447], tetrahydrochrysenes [Hwang, K.J., O'Neil, J.P. & Katzenellenbogen, J.A. (1992). 5,6,11,12-Tetrahydrochrysenes: Synthesis of rigid stilbene systems designed to be fluorescent ligands for the estrogen receptor. *J. Org. Chem.* 57, 1262-1271] etc.), and probably represent the filling of a preformed pocket of limited volume in the receptor by this substituent [Anstead, G.M., Carlson, K.E. & Katzenellenbogen, J.A. (1997) supra]. The principal difference in binding, however, is between the tetra-substituted imidazoles (**6b-d**, **12**, **17**) and the di- or tri-substituted imidazole (**3** and **6a**), the tetrasubstituted ones having much higher affinity. There is little difference in binding between imidazoles **12** and **17**, which have a different arrangement of nitrogen atoms in the heterocyclic core, but display their four substituents in an identical fashion. The overall low binding affinity of the imidazoles as a class might arise from the high inherent polarity of this heterocyclic system. The dipole moment for imidazole is very large, 5.56 D, and this may be unfavorable for binding to the estrogen receptor.

Table 4 shows the binding data for two thiazoles and oxazoles prepared. Although affinities are again very low, the more highly substituted thiazole again has the higher affinity (**22a** vs. **22b**). The oxazole **29** has undetectable affinity for ER. The isomer **31**, however, does have measurable though low binding. In contrast to imidazoles, thiazoles and oxazoles do not have very high dipole moments; so overall polarity is not likely to be the source of their low ER binding affinity, although heteroatom orientation appears to play a role (**29** vs. **31**). However, in the imidazole series, the compounds with the highest affinities were all tetrasubstituted. Since it is only possible to trisubstitute a thiazole or oxazole, this core structure may be unable to present sufficient peripheral substituents to afford ligands with good ER binding affinities.

The low binding affinities of the imidazoles, thiazoles and oxazoles may be, at least in part, due to their overall structure which is expected to be rather planar. It has been reported that good ligands for the estrogen receptor need to have some degree of "thickness" in the central portion of the ligand [41]. When alkyl substituents are added to either the imidazoles or thiazoles, their RBA increases. This increased binding could be due to an increase in steric bulk

around the central portion of the molecule, the result, in part, of a twisting of some of the aromatic substituents (see below) or to an increase in lipophilicity.

Durani et al. (1989) "Structure-activity relationship of antiestrogens: a study using triarylbutenone, benzofuran, and triarylfuran analogues as models for triarylethylenes and triarylpropenones" *J. Med. Chem.* 32:1700-1707 reported receptor affinity and biological activity data for several structural classes including triarylfurans.

Furans- RBA data and differential binding affinities for ERα and ERβ for several furans are given in Table 5 Furan **204**, for example, exhibits relatively high RBA. Several furans exhibit significant binding strength preference for ERα compared to ERβ. Furan **203**, for example, binds to ERα about 70-fold more strongly than it does to ERβ.

Pyrazoles and Isoxazoles – The RBA data for the 1,2-azoles are presented in Tables 6A-B. Immediately apparent is the relatively high binding affinity of pyrazoles **38b** and **38d**. An interesting comparison can be made among compounds **35a**, **35b**, **38a**, and **38b**. The disubstituted progenitor **35a** has very low affinity; addition of a third substituent, 1-phenyl in **35b** or 4-ethyl in **38b**, causes only a 2-fold or 3-fold increase in binding affinity, respectively. By contrast, addition of the fourth substituent (to give **38b**) causes either an 800- or 500-fold increase in binding affinity, respectively. Clearly, this is not additive behavior – two groups that each alone raise binding affinity 2- and 3-fold, together raise binding not 6-fold but 1500-fold. This suggests that high binding affinity is achieved when there is a detailed and proper match between the peripheral substituents and several subsites on the receptor. In the azole system, it appears that enhanced binding is associated with a tetrasubstituted ring. Consistent with this is the lower affinity of the isoxazole **41**, whose affinity is similar to the most closely related trisubstituted pyrazole **38a**.

There are other interesting trends in the pyrazole series: Replacement of the N-phenyl substituent (**38b**) with an N-benzyl group (**38c**) causes a significant 100-fold reduction in

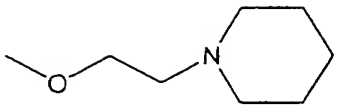
binding. Both of these compounds are tetrasubstituted pyrazoles. The decrease in binding affinity in **38b** vs. **38c** again suggests the need for a detailed match between ligand substituents and receptor subsites: the extra "kink" in the benzyl substituent in **38c** may be repositioning the peripheral substituents in a less favorable geometry. The addition of a hydroxyl group at the para position of the N-phenyl substituent (compound **38d** vs. **38b**) has only a minor effect on binding, indicating that polarity is well tolerated in this region of the receptor.

Differential ligand binding affinities for ERα and ERβ can be measured using purified preparations of human ERα and ERβ as described in Example 1B. Using this assay, Pyrazole compound **38b** was found to bind to ERα three-fold more strongly than to ERβ. This result indicates that certain ER ligands of this invention can exhibit differential ligand binding affinity to the different ER subtypes.

ER binding affinities of pyrazole isomers of core structure PA2 (Table 1) are given in Table 7. Overall, these pyrazole isomers have lower ER affinity (RBA) compared to pyrazoles of core structure PA1 by an average of about 2-fold. However, the structure-binding affinity pattern for both pyrazole isomers is quite similar. It is believed that these two core structure pyrazole isomers are binding in the same orientation in the ER binding pocket. Thus, it is possible to permute the position of heteroatoms in the azole ring without major effect on ER binding affinity provided that the peripheral substituents remain disposed with the same geometry and provided that one remains in the same azole series. The pyrazole isomers are compounds with equivalent dipole moment and polarities.

Pyrazoles with basic side groups

Table 8 presents ER binding affinity data for pyrazole of the indicated formula where one of R, R^2, R″or R‴is a cyclic amine group, i.e., a piperidinylethoxy group:



and otherwise R^2 is ethyl, and R is OH, R″and R‴are H or OH groups, as shown in Table 8. The presence of this basic side group has been associated with mixed agonist/antagonist activity. As indicated in Table 8, the pyrazole 301 in which the basic group is substituted at R‴ (with R^2 = ethyl, R and R″ both = OH) gives a very high affinity ER ligand.

RBA of compounds of structures disclosed herein as potential ER ligands either prepared by solution methods or preferably prepared by combinatorial synthetic methods can be readily determined using testing methods disclosed herein. Differential binding affinity of compounds herein can also be readily determined using methods described herein.

Cyclopentadienes- The relative ER binding affinity data of cyclopentadienes **230-237** are provided in Table 9. The ER binding affinities of the cyclopentadiene ligands are generally lower than those of pyrazoles, but exhibit similar patterns of binding affinity as a function of substituents. Cyclopentadiene **235** exhibits relative high ER binding affinity of 8.91%.

Pyridazines- All pyridazines (see, Table 13A) that have been assessed for binding to ER have exhibited no measurable binding affinity. The pyridazines are much more polar than the other 5- and 6-member ring compounds. It is believed that the high polarity of the core is detrimental to ligand binding to ER.

Pyrimidines-RBA values for several pyrimidines of structure PM4 are provided in Table 10. The binding affinities of these pyrimidines for ER are generally lower than those of the 5-membered ring ligands. Again, however, the compounds exhibit similar patterns of binding affinity as a function of substituents. In addition, pyrimidines of structure PM4 where R^2 =

methyl, R^1 and R^5 = pOH-phenyl and R^3 = $-CH_2-C_6H_4$ or R^3 = $-CH_2-C_6H_4$-pOH exhibited relatively low RBA of 0.032% and 0.013% respectively. The pyrimidine in which R3 = $-CH_2-C_6H_4$ exhibited no clear preference for binding to an ER subunit (ERα = 1.26% and ERβ = 0.696%). The pyrimidine in which R^3 = $-CH_2-C_6H_4$-pOH exhibited a preference for binding to ERα (ERα = 0.417% and ERβ = 0.076%).

A pyrimidine that can be characterized as having structure PM3 where R^1 and R^3 are pOH-phenyl groups, R^4 is a phenyl group and R^5 is ethyl exhibited a reasonable RBA of 1.00% with a clear preference for binding to ERα with ERα = 9.5% and ERβ = 3.24%.

Pyrazines- A mixture of two pyrazines regioisomers of structure PZ1 was assessed for relative ER binding affinity. The isomers where those where R^2 was ethyl, R^1 was pOH-phenyl and either R^4 or R^5 was p-OH-phenyl. RBA of 2.63% was measured for this mixture and a apparent preference for binding to ERα was observed (ERα = 7.94% and ERβ = 2.24%). A pyrazine of the same structure with R^2 = ethyl, and all of R^1, R^4 and R^5 = p-OH-phenyl was found to have a significantly lower RBA of 0.263%. This pyrazine exhibited a similar apparent preference for for binding to ERα with ERα = 7.41% and ERβ = 2.51%.

Quinoxalines- two mixtures containing quinoxaline regioisomers of structure QX2 (Table 2) were assessed for ER relative binding affinity (RBA) and binding affinity to the individual ER subunits. The pair of regioisomers, where R^2 = ethyl, R^1 = p-OH-phenyl with either R' = OH or R" = OH, exhibited a relatively low RBA of 0.20% with an apparent preference for binding to ERβ (ERα = 0.537% and ERβ = 0.933%). The pair of regioisomers, where R^2 = n-propyl, R^1 = p-OH-phenyl with either R' = OH or R" = OH, exhibited a much lower RBA of 0.014% and again exhibited a clear preference for binding to ERβ (ERα = 0.03% and ERβ = 0.224%).

Agonist/Antagonist Character of ER Ligands

Compounds are tested as ER agonists/antagonists in transcriptional activation assays in cells expressing ERα or ERβ. Cells are transfected with an expression plasmid for ERα or ERβ together with an estrogen-responsive reporter gene construct e.g., $(ERE)_3$-pS2-CAT, and treated with increasing concentrations of the test compound or with estradiol for comparison. Reporter gene expression is a measure of the capacity of ER complexed with various compounds to activate transcription, and it is followed as a function of concentration of the test compound. Potency and agonist character in activating transcription is measured relative to activation of the same system by estradiol. The ability of the test compound to inhibit transcriptional activation by increasing concentrations of estradiol is also measured as a function of test compound concentration. The ability of a test compound to inhibit transcriptional activation by estradiol is a measure of antagonist character and antagonist potency of the test compound.

Transcriptional activation can be assessed with ERα or ERβ and in different cells types. Using the $(ERE)_3$-pS2-CAT reporter, CAT activity is measured as a function of the concentration of added test compound (typically ranging from 10^{-12} - 10^{-6} molar) in the presence or absence of the known stimulator (estradiol, typically ranging from 10^{-12} - 10^{-6} molar). Agonist and/or antagonist character can be selective for ERα and ERβ. Assays can be performed, for example, in human endometrial cancer (HEC-1) cells, Chinese hamster ovarian (CHO) cells, and HeLa cells. Agonist/antagonist character can also be assessed with various promoters, e.g., the estrogen-responsive pS2 promoter, the simple TATA promoter, a non-consensus lactoferrin estrogen-responsive promoter, a heterologous thymidine kinase promoter and the complement C3 promoter which is an estrogen-responsive promoter that contains a non-consensus ERE.

The agonist/antagonist character of a given test compound relative to a selected ER ligand, e.g., estradiol, can be assessed using the transcriptional activation assays described. A given compound may be a pure agonist activating expression and exhibiting no transcriptional inhibition, a pure antagonist suppressing stimulation of expression by known activators and not stimulating transcription themselves or a mixed agonist/antagonist showing both types of

behavior. Test compounds may exhibit selectivity in potency, where a given test compound stimulates transcription at lower concentration through one ER subtype than through the other ER subtype. Test compounds may exhibit selectivity in that they stimulate transcription or inhibit expression to a greater degree through one or the other of ERα and ERβ. Test compounds can exhibit a different level of potency for activation compared to inhibition of stimulation of gene expression.

Figures 1A and B are graphs of transcriptional activation by ERα and ERβ, respectively, i response to pyrazole compound **38b** in HEC-1 cells using $(ERE)_3$-pS2-CAT. The figures plot CAT reporter activity as a function of the concentration of the ER ligand. Both figures also show the effect of estradiol (E2) on transcriptional activation by the ER subunits. Pyrazole **38b** is an ERα potency selective agonist compared to estradiol. The pyrazole exhibited a 120-fold higher potency in activating transcription via ERα than via ERβ. In contrast, estradiol exhibits significantly lower activation selectivity between ERα and ERβ. Similar ERα potency-selective character was observed for this pyrazole in other cell types and with other estrogen-responsive promoters. As noted above pyrazole compound **38b** was found to bind to ERα three-fold more strongly than to ERβ. Thus, differences in relative binding of the ligand does not fully account for the significantly higher (120-fold) selectivity for activation exhibited by the pyrazole with ERα compared that exhibited by the pyrazole with ERβ. These results suggest that factors beyond ligand-receptor interaction, such as receptor-coactivator interactions are likely important determinants of transcriptional potency.

Figure 2 is a graph of transcriptional activation by ERα (diamonds) and ERβ.(squares) in response to pyrazole 334 and pyrazole 336. Both of the pyrazoles assayed are potent in activating transcription under the assay conditions through ERα, but are weak or very weak transcriptional activators through ERβ. Both of these pyrazoles are ERα-potency selective agonists. Pyrazole 336 exhibits no activation through ERβ, even at the highest concentrations used. This pyrazole can be classified as an ERα-specific agonist. For both pyrazoles tested, the

difference in ERα and ERβ binding affinities parallels the observed potency selectivity or specificity.

The transcriptional activity profiles of pyrazole 301, which carries a basic piperidinylethoxy group, were examined for ERα and ERβ, respectively in HEC-1 cells as described in the Examples. Figures 3A and 3B are graphs of the transcriptional profiles (CAT activity) of pyrazole 301 for ERα and ERβ, respectively. Pyrazole 301 displayed no agonist activity on ERβ (Fig. 3B). However, on ERα this compound was a partial agonist, reaching an efficacy level nearly half that of estradiol at 1 nM (Fig. 3A). Interestingly, as the concentration of compound 301 increases, the ERα agonist activity returns to near basal levels. At high concentrations, pyrazole 301 acts as an antagonist through both ERα and ERβ., its potency as an antagonist through ERα being about 10-fold higher than through ERβ, which is consistent with its higher affinity for the ERα subtype (see Table 8). Pyrazole 301 is unusual, however, in that it exhibits a biphasic agonist-antagonist dose response through ERα. Many compounds exhibit partial agonist activity on ERα, and they are often more complete antagonists on ERβ than on ERα. However, typically, as the concentration of ligand increases, a constant level of efficacy is reached in assays of agonist and antagonist activity. Pyrazole 301, in contrast, demonstrates agonist activity up to nearly 50% that of estradiol, but its efficacy then decreases to only 10%.

The agonist character and antagonist character of compounds of structures disclosed herein as potential ER ligands either prepared by solution methods or preferably prepared by combinatorial synthetic methods can be readily determined using testing methods disclosed herein.

Pharmaceutical Compositions and Methods

This invention is also directed to pharmaceutically acceptable esters and salts of the ER ligands of various formulas and structures disclosed herein. Acid addition salts are prepared by contacting compounds having appropriate basic groups therein with an acid whose anion is generally considered suitable for human or animal consumption. Pharmacologically acceptable

acid addition salts include, but are not limited, to the hydrochloride, hydrobromide, hydroiodide, sulfate, phosphate, acetate, propionate, lactate, maleate, malate, succinate, and tartrate salts. All of these salts can be prepared by conventional means by reacting, for example, the selected acid with the selected basic compound. Base addition salts are analogously prepared by contacting compounds having appropriate acidic groups therein with a base whose cation is generally considered to be suitable for human or animal consumption. Pharmacologically acceptable base addition salts, include but are not limited to ammonium, amine and amide salts.

Pharmaceutically acceptable esters of compounds of this invention are prepared by conventional methods, for example by reaction with selected acids. Pharmaceutically acceptable esters include but are not limited to carboxylic acid esters RCOO-D (where D is a cationic form of a compound of this invention and where R is H, alkyl or aryl groups).

This invention is also directed to prodrugs and derivatives which on being metabolized will result in any of the ER ligands of this invention. For example, alkoxy or acetate groups can be metabolized to hydrogens. Labile substituents may be protected employing conventional and pharmaceutically acceptable protecting groups removable on metabolism. Pharmaceutically active compounds may be derivatized by conventional methods to provide for extended metabolic half-life, to enhance solubility in a given carrier, to provide for or facilitate slow-release or timed-release or enhance or affect other drug delivery properties.

Pharmaceutical compositions according to the present invention comprise one or more ER ligands of this invention in association with a pharmaceutically acceptable carrier or exipient adapted for use in human or veterinary medicine. The carrier is generally selected, as is known in the art for the particular application and should be compatible with the active ingredients. Such compositions may be prepared for use in conventional manner in admixture with one or more physiologically acceptable carriers or exipient. The compositions may optionally further contain one or more other therapeutic agents which may, if desired, be known ER ligands (agonists, antagonists and/or mixed agonist-antagonist as appropriate). ER ligands are present in

these pharmaceutical compositions in an amount or in a combined amount sufficient to elicit a measurable positive effect on a symptom or condition associated with an estrogen-dependent disorder on administration to an individual suffering from the symptom or disorder.

The ER ligands according to the invention may be formulated for oral, buccal, parenteral, topical or rectal administration. In particular, the ER ligands according to the invention may be formulated for injection or for infusion and may be presented in unit dose form in ampules or in multidose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use. Aqueous vehicles can be provided with pH control agents, electrolyte control or agents that enhance solubility of the active ingredients in the vehicle.

The pharmaceutical compositions according to the invention may also contain other active ingredients such as antimicrobial agents, or preservatives.

In general, pharmaceutical compositions of this invention can contain from 0.001-99% (by weight) of one or more of the ER ligands disclosed herein. ER ligands may be provided as pure regioisomers or as a mixture of regioisomers. Analogously, ER ligands may be provided as a mixture of enantiomeric forms or as a purified enantiomer.

The invention further provides a process for preparing a pharmaceutical composition which comprises bringing a ER ligand of the invention into association with a pharmaceutically acceptable exipient or carrier. The carrier or exipient being selected as is known in the art for compatibility with the desired means of administration, for compatibility with the selected ER ligands and to minimize detrimental effects to the patient.

For administration by injection or infusion, the daily dosage as employed for treatment of an adult human of approximately 70 kg body weight will range from 0.2 mg to 10 mg, preferably 0.5 to 5 mg, which can be administered in 1 to 4 doses, for example, depending on the route of administration and the clinical condition of the patient. These formulations also include formulations in dosage units. This means that the formulations are present in the form of a discrete pharmaceutical unit, for example, as tablets, dragees, capsules, caplets, pills, suppositories or ampules. The active compound content of each unit is a fraction or a multiple of an individual dose. The dosage units can contain, for example, 1, 2, 3 or 4 individual doses for 1/2, 1/3 or 1/4 of an individual dose. An individual dose preferably contains the amount of active compound which is given in one administration and which usually corresponds to a whole, one half, one third or one quarter of a daily dose.

The magnitude of a prophylactic or therapeutic dose of a particular compound will, of course, vary with the nature of the severity of the condition to be treated, the particular ER ligand compound and its route of administration. It will also vary according to the age, weight and response of the individual patient.

The compounds of the present invention are preferably formulated prior to administration. The present pharmaceutical formulations are prepared by known procedures using well-known and readily available ingredients. In making the compositions of the present invention, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material which acts as a vehicle, exipient or medium for the active ingredient. The compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing for example up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders.

Some examples of suitable carriers, exipient, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.5 to about 150 mg, more usually about 0.1 to about 10 mg, of the active ingredient. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

As a pH adjusting reagent for preparing the pharmaceutical composition, any allowed for preparing medicines can be used, including but not limited to hydrochloric acid-sodium hydroxide, acetic acid-sodium acetate, glycine-sodium chloride-hydrochloric acid, potassium dihydrogenphosphate-disodium hydrogenphosphate, potassium hydrogenphthalate-sodium hydroxide, sodium secondary citrate-hydrochloric acid, sodium dihydrogen-phosphate-disodium hydrogenphosphate, sodium dihydrogenphosphate-dipotassium hydrogen-phosphate, potassium dihydrogenphosphate-dipotassium hydrogenphosphate, tartaric acid-sodium tartrate, lactic acid-sodium lactate, sodium barbital-sodium acetate-hydrochloric acid, succinic acid-boric acid, potassium primary citrate-sodium hydroxide, sodium primary citrate-borax, disodium hydrogenphosphate-citric acid, sodium acetate-hydrochloric acid, glutamic acid-sodium hydroxide, and aspartic acid-sodium hydroxide. Among them, hydrochloric acid-sodium hydroxide, acetic acid-sodium acetate, glycine-sodium chloride-hydrochloric acid, tartaric

acid-sodium tartrate, lactic acid-sodium lactate, sodium acetate-hydrochloric acid, glutamic acid-sodium hydroxide, and aspartic acid-sodium hydroxide.

This invention is further directed to therapeutic methods employing the ER ligands of this invention and pharmaceutical compositions containing them in the treatment of estrogen-dependent or estrogen-related disorders. These methods comprise a step of administering to a patient having the disorder or symptoms thereof a pharmaceutical composition comprising one or a mixture of the ER ligands of this invention where the ER ligand or mixture of ligands is present in the composition at a level or a combined level sufficient to effect a positive biological response. The present invention provides ER ligands that can be used in place of or in combination with currently known pharmaceuticals active in estrogen-dependent or estrogen-related disorders. Certain ER ligands of this invention and certain ER ligands identified by the combinatorial synthetic methods and selective assays described herein can exhibit improved properties (enhanced activity and/or decreased undesired side-effects) for treatment of estrogen-dependent and estrogen-responsive disorders.

The ER ligands of this invention are useful in vitro and/or in vivo for selective activation or repression of expression, dependent upon the agonist or antagonist nature of the ligand or its potency, of a gene regulated by ER. Gene activation or repression can be selective with respect to subtype of ER (e.g., ERα or ERβ), or variant of ER (e.g., splice variant forms, truncated or processed forms, covalently modified forms, etc.).

The ER ligands of this invention are also useful in vitro and/or in vivo for selective regulation of cellular activities under the control of ER. Cellular activities may be regulated in a variety of ways by ER, subtypes of ER or variants of ER, e.g., up or down regulation of a given cellular process. Regulation is selective with respect to subtype of ER (e.g., ERα or ERβ), or variant of ER (e.g., splice variant forms, truncated or processed forms, covalently modified forms, etc.). Cellular activities that may be regulated include both genomic (related to gene expression) or non-genomic activities (not directly related to gene expression, e.g., such as

regulation of calcium flux, particularly in bone cells, hormone release, particularly prolactin release from pituitary cells, etc.).

The subtype-selective ER ligands of this invention can also be of general use in the investigation of ER and its functions. These ligands can be employed to better understand structure and conformation of ER (both subtypes) and to elucidate how ER subtypes interact with other molecules and to relate structure, conformation and interaction with other molecules to ER function.

Agents that can act selectively to stimulate or inhibit estrogen action through the individual ER subtypes can be useful in achieving selective regulation of specific responses and specific tissues. For example, ERβ appears responsible for mediating the beneficial effects of estrogens in suppressing vascular cell overgrowth in response to blood vessel injury. Therefore, an ER ligand that antagonizes only ERβ-mediated responses should block this response without blocking desired responses to estrogens that are mediated by ERα, such as maintenance of a favorable profile of blood lipids. Preferred ER ligands of this invention which exhibit selective interaction with ER subtypes can be employed to selectively stimulate or inhibit estrogen action. References that relate to tissue distribution of ER subtypes include: Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson J, Nilsson S 1998 Differential response of estrogen receptor a and estrogen receptor β to partial estrogen agonists/antagonists. Mol Pharmacol 54:105-112; Couse JF, Lindsey J, Grandien K, Gustafsson J-A, Korach KS 1997 Tissue distribution and quantitative analysis of estrogen receptor-alpha and estrogen receptor-beta messenger ribonucleic acid in the wild type and ER-alpha knockout mouse. Endocrinology 138:4613-4621; Dotzlaw H, Leygue E, Watson PH, Murphy LC 1997 Expression of estrogen receptor-β in human breast tumors. J. Clin. Endocrinol. Metab. 82:2371-2377; Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson J 1998 Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 139:4252-4263; Kuiper GGJM, Gustafsson J-A 1997 The novel estrogen receptor-beta subtype: potential role in the cell- and promoter-specific actions of estrogens and anti-estrogens. FEBS Letters

410:87-90; Katzenellenbogen BS, Korach KS 1997 Editorial: A new actor in the estrogen receptor drama-Enter ER-β. Endocrinology 138:861-862; Montano MM, Jaiswal AK, Katzenellenbogen BS 1998 Transcriptional regulation of the human quinone reductase gene by antiestrogen-liganded estrogen receptor-α and estrogen receptor-β. J Biol Chem 273:25443-25449; Register TC, Shively CA, Lewis CE 1998 Expression of estrogen receptor alpha and beta transcripts in female monkey hippocampus and hypothalamus. Brain Res 788:320-322; Register TC, Adams MR 1998 Coronary artery and cultured aortic smooth muscle cells express mRNA for both the classical estrogen receptor and the newly described estrogen receptor beta. J Steroid Biochem Molec Biol 64:187-191).

The estrogen subtypes, ERα and ERβ, are the products of two different genes. However, variant forms of both ER subtypes are known. ERβ variants having different N-terminal lengths that correspond to different transcriptional start sites are known (McInerney EM, Weiss KE, Sun J, Mosselman S, Katzenellenbogen BS 1998 Transcription activation by the human estrogen receptor subtypeβ (ERβ) studied with ERβ and ERα receptor chimeras. Endocrinology 139:4513-4522; Montano MM, Jaiswal AK, Katzenellenbogen BS 1998 Transcriptional regulation of the human quinone reductase gene by antiestrogen-liganded estrogen receptor-α and estrogen receptor-β. J Biol Chem 273:25443-25449). In addition, gene transcripts with deleted exons and alternate exon splicing, which may be translated into proteins, are known. These variant ER forms can have different transcription regulating activities, and can respond differently to different ER ligands (Chaidarun S, Alexander J 1998 A tumor-specific truncated estrogen receptor splice variant enhances estrogen-stimulated gene expression. Mol Endocrinol 12:1355-1366; Leygue ER, Watson PH, Murphy LC 1996 Estrogen receptor variants in normal human mammary tissue. J Natl Cancer Inst 88:284-290; Miksicek RJ, Lei Y, Wang Y 1993 Exon skipping gives rise to alternatively spliced forms of the estrogen receptor in breast tumor cells. Breast Cancer Res Treat 26:163-174; Pfeffer U, Fecarotta E, Vidali G 1995 Coexpression of multiple estrogen receptor variant messenger RNAs in normal and neoplastic breast tissue and in MCF-7 cells. Cancer Res 55:2158-2165; Zhang QX, Borg A, Fuqua SAW 1993 An exon 5

deletion variant of the estrogen receptor frequently co-expressed with wild-type estrogen receptor in human breast cancers. Cancer Res 53:5882-5884).

Various mutant forms of ERs have been characterized, and some of these show different responses to ER ligands (Wrenn CK, Katzenellenbogen BS 1993 Structure-function analysis of the hormone binding domain of the human estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast. J Biol Chem 268:24089-24098; Montano MM, Ekena KE, Krueger K, Keller AL, Katenellenbogen BS 1996 Human estrogen receptor ligand activity inversion mutants: Receptors that interpret antiestrogens as estrogens and estrogens as antiestrogens and discriminate among different antiestrogens. Mol Endocrinol 10:230-242). ERs can be covalently modified by post-transcriptional events, such as phosphorylation, acetylation, and glycosylation. These modifications can also alter ER responsiveness to different ER ligands (Le Goff P, Montano MM, Schodin DJ, Katzenellenbogen BS 1994 Phosphorylation of the human estrogen receptor: Identification of hormone-regulated sites and examination of their influence on transcriptional activity. J Biol Chem 269:4458-4466; Kato SH, Endoh Y, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masuchige S, Gotoh Y, Nishida E, Kawashima H, Metzger D, Chambon P 1995 Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science 270:1491-1494).

Some of the actions of estrogens appear to be non-genomic, and may involve action through ERs in the cell membrane. Examples of such responses are stimulation of calcium flux regulation in bone cells and prolactin release from pituitary cells (Lieberherr M, Grosse B, Kachkache M, Balsan S 1993 Cell signaling and estrogens in female rat osteoblasts: A possible involvement of unconventional nonnuclear receptors. J Bone Miner Res 8:1365-1376; Marino M, Pallottini V, Trentalance A 1998 Estrogens cause rapid activation of IP3-PKC-a signal transduction pathway in HEPG2 cells. Biochem Biophys Res Commun 245:254-258; Mermelstein PG, Becker JB, Surmeier DJ 1996 Estradiol reduces calcium currents in rats neostriatal neurons via a membrane receptor. J Neuroscience 16:595-604; Pappas TC, Gametchu B, Watson CS 1995 Membrane estrogen receptors identified by multiple antibody labeling and

impeded-ligand binding. FASEB J 9:404-410; Pappas TC, Gametchu B, Yannariello-Brown J, Collins TJ, Watson CS 1994 Membrane estrogen receptors in GH3/B6 cells are associated with rapid estrogen-induced release of prolactin. Endocrine 2:813-822; Wehling M 1997 Specific, nongenomic actions of steroid hormones. Annu Rev Physiol 59:365-393; Zheng J, Ramirez VD 1997. Demonstration of membrane estrogen binding proteins in rat brain by ligand blotting using a 17b-estradiol-[^{125}I] bovine serum albumin conjugate. J Steroid Biochem Molec Biol 62:327-336). Although it is not known, as yet, which ER subtypes are important in regulating non-genomic responses, subtype-selective ER ligands may enable regulation of these responses in a tissue and cell selective manner.

Methods for selective regulation of cellular activities through ER employing the ER ligands of this invention can be used with variant, mutant and modified ERs as described herein and as known in the art. The interaction of ER ligands of this invention with variant, mutant and modified ERs can be assessed as described herein for ER.

The following examples are provided to further illustrate the invention and are in no way intended to limit the scope of the invention.

EXAMPLES

Example 1:

Measurement of relative ER Binding Affinities

A.

ER ligand binding assays can be performed as previously reported [Katzenellenbogen, J.A. et al. (1977) "Estrogen photoaffinity labels. 1. Chemical and radiochemical synthesis of hexestrol diazoketone and azide derivatives; photochemical studies in solution." Biochemistry 16:1964-1970] using lamb and/or rat uterine cytosol diluted to approximately 1.5 nM of receptor, which was incubated with buffer of several concentrations of unlabeled competitor together with

10 nM [3H]estradiol for 18-24 hours. Free ligand was removed by adsorption to dextran-coated charcoal. Unlabeled competitors were prepared in 1:1 DMF:TEA to ensure solubility.

B.

Differential ligand binding affinities for ERα and ERβ can be determined by competitive radiometric binding assays using 10 nM [^{3}H]estradiol as tracer, and hydroxylapatite to adsorb bound receptor-ligand complex, as described previously [Carlson, K.E. et al. (1997) "Altered ligand binding properties and enhanced stability of a constitutively active estrogen receptor: evidence that an open-pocket conformation is required for ligand interaction," Biochemistry 36:14897-14905]. Differential assays are performed using purified preparations of human ERα (amino acids 304-554) and ERβ (203-452) ligand binding domains expressed in *E. coli* or using full length ERα and ERβ expressed in *Baculovirus* (commerically available).

Example 2:

Cell assays for ER Ligand Activity

Compounds are tested as ER agonists/antagonists in transcriptional activation assays in cells expressing ERα or ERβ. Cells are transfected with an expression plasmid for ERα or ERβ together with an estrogen-responsive reporter gene construct e.g., $(ERE)_3$-pS2-CAT, and treated with increasing concentrations of the test compound or with estradiol for comparison. Reporter gene expression is a measure of the capacity of ER complexed with various compounds to activate transcription, and it is followed as a function of concentration of the test compound. Potency and agonist character in activating transcription is measured relative to activation of the same system by estradiol. The ability of the test compound to inhibit transcriptional activation by increasing concentrations of estradiol is also measured as a function of test compound concentration. The ability of a test compound to inhibit transcriptional activation by estradiol is a measure of antagonist character and antagonist potency of the test compound.

Transcriptional activation can be assessed with ERα or ERβ and in different cells types. Using the $(ERE)_3$-pS2-CAT reporter, CAT activity is measured as a function of the

concentration of added test compound (typically ranging from 10^{-12} - 10^{-6} molar) in the presence or absence of the known stimulator (estradiol, typically ranging from 10^{-12} - 10^{-6} molar). Agonist and/or antagonist character can be selective for ERα and ERβ. Assays can be performed, for example, in human endometrial cancer (HEC-1) cells, Chinese hamster ovarian (CHO) cells, and HeLa cells. Agonist/antagonist character can also be assessed with various promoters, e.g., the estrogen-responsive pS2 promoter, the simple TATA promoter, a non-consensus lactoferrin estrogen-responsive promoter, a heterologous thymidine kinase promoter and the complement C3 promoter which is an estrogen-responsive promoter that contains a non-consensus ERE.

The agonist/antagonist character of a given test compound relative to a selected ER ligand, e.g., estradiol, can be assessed using the transcriptional activation assays described. A given compound may be a pure agonist activating expression and exhibiting no transcriptional inhibition, a pure antagonist suppressing stimulation of expression by known activators and not stimulating transcription themselves or a mixed agonist/antagonist showing both types of behavior. Test compounds may exhibit selectivity in potency, where a given test compound stimulates transcription at lower concentration through one ER subtype than through the other ER subtype. Test compounds may exhibit selectivity in that they stimulate transcription or inhibit expression to a greater degree through one or the other of ERα and ERβ. Test compounds can exhibit a different level of potency for activation compared to inhibition of stimulation of gene expression.

Pyrazole compound **38b** was found to be an ERα potency selective agonist compared to estradiol when assayed in HEC-1 cells using $(ERE)_3$-pS2-CAT. It exhibited a 120-fold higher potency in activating transcription via ERα than via ERβ. In contrast, estradiol exhibits significantly lower activation selectivity between ERα and ERβ. Similar ERα potency-selective character was observed for this pyrazole in other cell types and with other estrogen-responsive promoters. Pyrazole compound **38b** was found to bind to ERα three-fold more strongly than to ERβ. Thus, differences in relative binding of the ligand does not fully account for the

significantly higher (120-fold) selectivity for activation exhibited by the pyrazole with ERα compared that exhibited by the pyrazole with ERβ. These results suggest that factors beyond ligand-receptor interaction, such as receptor-coactivator interactions are likely important determinants of transcriptional potency.

CHEMICALS, MATERIALS, AND PLASMID CONSTRUCTIONS

Cell culture media were purchased from GIBCO (Grand Island, NY). Calf serum was from Hyclone Laboratories (Logan, UT) and fetal calf serum was from Atlanta Biologicals (Atlanta, GA). ^{14}C-Chloramphenicol (50-60 Ci/mmol) and [^{3}H]E_2 were from DuPont, NEN Research Products (Boston, MA). The expression vector for human ERα (pCMV5-hER) was constructed previously as described (Wrenn, C.D. and Katzenellenbogen, B.S. (1993), "Structure-function analysis of the hormone binding domain of the human estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast," J. Biol. Chem. **268**:24089-24098). The expression vector pCMV5-ERβ was constructed by inserting the full-length cDNA encoding human ERβ (530) residues, pNGV1-ERβ (Mosselmen et al. (1996) supra) and including the additional 53 N-terminal amino acids as found in Genebank accession number AF 051427), into the BamH1 site of pCMV5. The estrogen responsive reporter plasmids were $(ERE)_3$-pS2-CAT, constructed as described previously (Kraus, W.L. et al. (1995), "Ligand-dependent, transcriptionally productive association of the amino-and carboxyl-terminal regions of a steroid hormone nuclear receptor," Proc. Natl. Acad. Sci. USA 92:12314-12318), $(ERE)_2$-TATA-CAT [Wrenn, C.D. and Katzenellenbogen, B.S. (1993), "Structure-function analysis of the hormone binding domain of the human estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast," J. Biol. Chem. **268**:24089-24098], C3-Ti-LUC, which contains -1030 to +58 of the human complement C3 promoter fused to the firefly luciferase reporter gene (Norris, J.D. et al. (1996), "Identification of the sequences within the human complement 3 promoter required for estrogen responsiveness provides insight into the mechanism of tamoxifen mixed agonist activity," Mol. Endocrinol. **10**:1605-1616), and lactoferrin ERE-tk-CAT, which contains 2 copies of the non-consensus lactoferrin ERE fused to the thymidine kinase promoter and CAT reporter gene. The plasmid pCH110 (Pharmacia, Piscataway, NJ) or pCMVβ

(Clontech, Palo Alto, CA) which contains the β-galactosidase gene, was used as an internal control for transfection efficiency. Expression vectors employed herein are commerically available or available through routine preparations using published information.

Cell culture and transient transfections

Human endometrial cancer (HEC-1) cells, chinese hamster ovary (CHO) cells and HeLa cells are maintained in culture and transfected as described (Wrenn, C.D. and Katzenellenbogen, B.S. (1993), "Structure-function analysis of the hormone binding domain of the human estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast," J. Biol. Chem. **268**:24089-24098; Montano, M.M. et al. (1995), "The carboxyl-terminal F domain of the human estrogen receptor: role in the transcriptional activity of the receptor and the effectiveness of antiestrogens as estrogen antagonists," Mol. Endocrinol. **9**:814-825; McInerney, E.M. and Katzenellenbogen, B.S. (1996), "Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation," J. Biol. Chem. **271**:24172-24178). Transfection of HEC-1 cells in 60-mm dishes utilizes 0.4 ml of a calcium phosphate precipitate containing 2.5 μg of the reporter gene plasmid, 100 ng of ER expression vector, and carrier DNA to a total of 5 μg DNA. CAT or luciferase activity, normalized for the internal control β-galactosidase activity, is assayed as described (Montano, M.M. et al. (1995), "The carboxyl-terminal F domain of the human estrogen receptor: role in the transcriptional activity of the receptor and the effectiveness of antiestrogens as estrogen antagonists," Mol. Endocrinol. **9**:814-825; McInerney, E.M. and Katzenellenbogen, B.S. (1996), "Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation," J. Biol. Chem. **271**:24172-24178).

Example 3:

Chemical Syntheses

General Methods

All reactions using water- or air-sensitive reagents were conducted under an Ar atmosphere with dry solvents. Solvents were distilled under N_2 as follows: CH_2Cl_2 from CaH_2, THF from sodium benzophenone ketyl, DMF from $MgSO_4$, and Hexanes from $CaSO_4$. Triethyl amine was distilled over CaH_2. All other reagents were purchased from commercial suppliers and used without further purification. Reactions were all monitored by TLC, performed on 0.25 mm silica gel glass plates containing F-254 indicator. Visualization on TLC was achieved by UV light (254 nm), iodine vapors, or phosphomolybdic acid indicator. Flash chromatography was performed using Woelm 32-63 μm silica gel packing unless otherwise noted.

^1H NMR and ^{13}C NMR spectra were recorded on a Varian U400, Varian U500 or Varian INOVA 750. Electron ionization (EI) spectra were obtained using a Finnigan-MATCH5 spectrometer at 70 eV. Fast atom bombardment (FAB) were recorded on a VG ZAB-SE spectrometer. High pressure liquid chromatography (HPLC) was performed on a SpectraPhysics P100 solvent delivery system with ultraviolet detection at 254 nm. Elemental analysis was performed by the Microanalytical Service Laboratory at the University of Illinois.

Compound numbers listed refer to those in the Schemes.

General Demethylation Procedure using BBr_3. To a stirring solution of the methyl-protected heterocycle (1 eq.) in CH_2Cl_2 at -78 °C was added a solution of BBr_3 (4-5 eq.) as a 1N solution in CH_2Cl_2. The reaction were allowed to warm to room temperature and stirred for 18 h. After quenching with H_2O, the layers were separated and the aqueous layer extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to afford the crude phenols. Flash chromatography afforded the demethylated products.

General Demethylation Procedure using $BF_3 \cdot SMe_2$. To a stirring solution of the methyl protected heterocycle (1 eq.) in CH_2Cl_2 (8 mL) at room temperature was added $BF_3 \cdot SMe_2$ complex (75 eq.). After stirring for 24 h, solvent and excess reagent were evaporated under nitrogen stream in hood. Residue was taken up in EtOAc and washed with H_2O and sat. NaCl.

Organic extract was dried over Na_2SO_4, filtered and solvent removed under reduced pressure. The resulting residue was purified though a silica plug, eluting with EtOAc. Solvent evaporation afforded the deprotected products.

Imidazoles

4,5-Di(4-methoxyphenyl)-1H-imidazole (**2**). To 4,4'-dimethoxybenzil (**1**) (2.0g, 7.4 mmol) and *p*-formaldehyde (1.0 g, 11.1 mmol) was added formamide (50 mL). The bright yellow suspension was heated to reflux (220°C) for 2 h. The reaction mixture was then cooled to room temperature then to 0°C. The crystals that formed were filtered and recrystallized from EtOAc to afford **2** (2.4 g, 86%). mp 183-184°C (lit [16] mp 183-184°C);

4,5-Di(4-hydroxyphenyl)-1H-imidazole (**3**). Imidazole **2** (100 mg, 0.35 mmol) afforded **3** (52 mg, 59%) by the general BBr_3 demethylation procedure.

2,4,5-Tri(4-methoxyphenyl)-1H-imidazole (**4**). A suspension of 4,4'-dimethoxybenzil (**1**) (4.0 g, 15 mmol) and *p*-anisaldehyde (20 mL, 164 mmol) and formamide (100 mL) was heated to reflux (220°C) for 2 h, during which time the reaction mixture became homogeneous. The reaction was then cooled to 0°C and the precipitated product **4**, was filtered. The light yellow powder was recrystallized from MeOH/H_2O to afford 3.80 g of **4** [Hayes, J.F., Mitchell, M.B. & Wicks, C. (1994), "A novel synthesis of 2,4,5-triarylimidazoles," *Heterocycles* **38**, 575-585] (66%). mp 89-91°C (lit [Hayes, J.F., Mitchell, M.B. & Wicks, C. (1994), "A novel synthesis of 2,4,5-triarylimidazoles," *Heterocycles* **38**, 575-585] mp 88-94°C).

General N-Alkylation Procedure for Imidazoles. A solution of imidazole **4** (200 mg, 0.52 mmol) in THF (10 mL) and DMF (1.5 mL) was cooled to 5°C. NaH (31 mg, 0.78 mmol) was added as 60% dispersion in mineral oil. The reaction mixture was warmed to room temperature for 1 h and respective alkyl halide (0.04 mL, 0.62 mmol) was added. The resulting suspension was heated to reflux for 12 h, then cooled to room temperature. The light precipitate was filtered

and the filtrate was concentrated under vacuum to a yellow solid which was flashed on silica (30% EtOAc/Hexanes) to afford alkylated products **5b-d** 1-Ethyl-2,4,5-tri(4-methoxyphenyl)-imidazole (**5b**), 1-Propyl-2,4,5-tri(4-methoxyphenyl)-imidazole (**5c**), 1-Butyl-2,4,5-tri(4-methoxyphenyl)-imidazole (**5d**) in 80-90% yields.

2,4,5-Tri(4-hydroxyphenyl)-1H-imidazole (**6a**). According to the general BBr_3 demethylation procedure above, imidazole **4** (3.0 g, 7.8 mmol) afforded **6a** as a green-orange solid that darkened upon exposure to air (1.8 g, 68%). mp 203-205 °C.

1-Ethyl-2,4,5-tri(4-hydroxyphenyl)-imidazole (**6b**). According to the general BBr_3 demethylation procedure above, imidazole **5b** (185 mg, 0.46 mmol) afforded **5b** (107 mg, 62%). mp 150-153 °C;

1-Propyl-2,4,5-tri(4-hydroxyphenyl)-imidazole (**6c**). According to the general BBr_3 demethylation procedure above, imidazole **5c** (170 mg, 0.40 mmol) afforded **6c** (86 mg, 55%). mp 172-175 °C.

1-Butyl-2,4,5-tri(4-hydroxyphenyl)-imidazole (**6d**). According to the general BBr_3 demethylation procedure above, imidazole **5d** (190 mg, 0.43 mmol) afforded **6d** (78 mg, 46 %). mp 153-155 °C (dec).

1-Ethyl-2,5-(4-methoxyphenyl)-4-phenyl imidazole (**11**). Azido-ketone **9** (50.0 mg, 0.187 mmol) and imine **10** (92.0 mg, 0.564 mmol) were dissolved in THF (15 mL). Et_3N (29.0 µL, 0.208 mmol) was added via syringe and reaction stirred at room temperature for 48 h. The reaction mixture was then poured into H_2O and extracted with CH_2Cl_2, organic fractions were pooled, dried over Na_2SO_4, filtered and solvent removed under reduced pressure. The intermediate, 2,5-dihydro-2-hydroxyimidazole, used in next step without further purification or characterization, was taken up CH_2Cl_2 (10 mL). Solution was cooled to 0 °C and TFA (14.4 µL, 0.187 mmol) was added via syringe. Reaction stirred at 0 °C for 36 h. The mixture was diluted

with CH_2Cl_2 (10 mL) and washed with H_2O, sat. $NaHCO_3$, and sat. NaCl successively. Organic fraction was dried over Na_2SO_4, filtered and solvent removed under reduced pressure. Purification by flash column chromatography (1:2 EtOAc:Hexanes) and recrystallization from CH_2Cl_2/Hexanes afforded imidazole **11** as a white solid (24.6 mg, 34% yield from azide **9**).

1-Ethyl-2,5-(4-hydroxyphenyl)-4-phenyl imidazole **(12).** Imidazole **11** (12.0 mg, 0.031 mmol) was demethylated according to the general $BF_3 \cdot SMe_2$ procedure to afford imidazole **12** as an off-white powder (10.6 mg, 95%).

5-Ethyl-1,4-(4-methoxyphenyl)-2-phenyl imidazole **(16).** Keto-amide **15** (110.0 mg, 0.273 mmol) and ammonium acetate (105.0 mg, 1.362 mmol) were heated to reflux in acetic acid (10 mL) for 48 h. Acetic acid was removed under reduced pressure, resulting residue was taken up in EtOAc, washed with sat. $NaHCO_3$, H_2O, and sat. NaCl. Organic extracts were dried over Na_2SO_4, filtered and solvent removed. Product was purified by flash column chromatography (1:4 EtOAc:Hexanes) and recrystallization from CH_2Cl_2/Hexanes to give imidazole **16** as a white solid (25.7 mg, 25%).

5-Ethyl-1,4-(4-hydroxyphenyl)-2-phenyl imidazole **(17).** Imidazole **16** (25.0 mg, 0.065 mmol) was demethylated as outlined in general $BF_3 \cdot SMe_2$ procedure above to give deprotected imidazole **17** as an off-white powder (20.2 mg, 87%).

Thiazoles

2,4-Di(4-methoxyphenyl)-thiazole **(21a).** A suspension of thioamide **19** (1.3 g, 7.9 mmol) and α-bromo-4'-methoxy-acetophenone **(20)** (1.8 g, 7.9 mmol) in DMF (10 mL) was heated to reflux for 1h, until it became homogeneous. The heat was removed and the reaction was stirred for 15 h at room temperature. The reaction mixture was poured into H_2O (50 mL) and the solid precipitate was filtered to afford crude **21a**. Recrystallization from CH_3NO_2 afforded pure **21a** as light yellow crystals (1.8 g, 81%).

5-Ethyl-2,4-di(4-methoxyphenyl)-thiazole (**21b**). A suspension of thioamide **19** (975 mg, 5.8 mmol) and α-bromo-4'-methoxy-butyrophenone (**13**) (1.5 g, 5.8 mmol) in DMF (10 mL) was heated to reflux for 4 h, until it became homogeneous. The heat was removed and the reaction was poured into H_2O (50 mL). The water was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with sat. LiCl (10 mL), then brine (10 mL). After drying over $MgSO_4$, the reaction mixture was filtered, and concentrated to a yellow powder. Flash chromatography (10% EtOAc/Hexanes) afforded **21b** as a light yellow powder (1.1 g, 51%).

2,4-Di(4-hydroxyphenyl)-thiazole (**22a**). Thiazole **21a** (1.0 g, 3.6 mmol) was demethylated using BBr_3 as outlined in the general procedure above to afford **22a** (430 mg, 45%). mp 218-221 °C;

2,4-Di(4-hydroxyphenyl)-5-ethyl-thiazole (**22b**). Thiazole **21b** (1.0 g, 2.7 mmol) was demethylated according to the general BBr_3 procedure to afford **22b** (460 mg, 58%). mp 246-247 °C;

Oxazoles

2,4-(4-Methoxyphenyl)-5-phenyl oxazole (**28**). Azido-ketone **27** (0.18 g, 0.673 mmol) and *p*-anisaldehyde (0.25 mL, 2.05 mmol) were dissolved in THF (15 mL). Et_3N (94.0 μL, 0.674 mmol) was added via syringe and reaction stirred at room temperature for 48 h. The reaction mixture was then poured into H_2O and extracted with CH_2Cl_2, organic fraction was dried over Na_2SO_4, filtered and solvent removed under reduced pressure. Resulting intermediate 2,5-dihydro-5-hydroxyoxazole, used in next step without further purification or characterization, was taken up CH_2Cl_2 (10 mL). Solution was cooled to 0 °C and TFA (54.0 μL, 0.701 mmol) was added via syringe. Reaction stirred at 0 °C for 36 h. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with H_2O, sat. $NaHCO_3$, and sat. NaCl successively. Organic extracts were combined, dried over Na_2SO_4, filtered and solvent removed under reduced pressure . Purification by flash column chromatography (1:2 EtOAc:Hexanes) and recrystallization from CH_2Cl_2/Hexanes afforded oxazole **28** as a white solid (72.4 mg, 30% yield from azide **27**). mp

125-128 °C (lit. [Strzybny, P.P.E., van ES, T. & Backeberg, O.G. (1969), "Reaction of α-acyloxyketones with ammonium acetate," *J. South African Chem. Inst.* 22, 158-164] mp 126-127 °C);

2,4-(4-Hydroxyphenyl)-5-phenyl oxazole (**29**). Oxazole **28** (22.0 mg, 0.062 mmol) was demethylated according to the general $BF_3 \cdot SMe_2$ procedure above to give deprotected oxazole **29** as an off-white powder (18.8 mg, 93%).

2,5-(4-Methoxyphenyl)-4-phenyl oxazole (**30**). A solution of bromo-ketone **26** (87.0 mg, 0.285 mmol) and *p*-methoxybenzamide (43.0 mg, 0.285 mmol) in toluene was heated to reflux for 36 h. Toluene was removed under reduced pressure and resulting residue purified by flash column chromatography (1:4 EtOAc:Hexanes). Recrystallization of desired product from CH_2Cl_2/Hexanes afforded oxazole **30** as a colorless solid (52.9 mg, 52%). mp 147-149°C;

2,5-(4-Hydroxyphenyl)-4-phenyl oxazole (**31**). Oxazole **30** (22.0 mg, 0.062 mmol) was demethylated according to the general $BF_3 \cdot SMe_2$ procedure above to give deprotected oxazole **31** as an off-white powder (18.1 mg, 89%).

General Procedure for Pyrazole Synthesis. A suspension of diketone (1 eq.) and appropriate hydrazine hydrochloride (3-5 eq.) in a 3:1 mixture DMF:THF was heated to reflux for 16-24 h with reaction progress being monitored by TLC for disappearance of starting material. The reaction mixtures was cooled to room temperature and poured into iced sat. LiCl solution (10 mL) and EtOAc (10 mL). The layers were separated and the organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography (EtOAc/Hexanes systems) afforded the pyrazoles.

3,5-Di(4-methoxyphenyl)-1H-pyrazole (**34a**). Diketone **33** (91 mg, 0.32 mmol) and hydrazine (0.1 mL, 3.2 mmol) were reacted as outlined in general pyrazole procedure to afford **34a** [van Steenis, J. (1946), "The nitration of dianisoylmethane and p-methoxydesoxybenzoin," *Chem. Ber.* , 29-46] as an off-white solid (32.6 mg, 38%). mp 172-175°C (lit [van Steenis, J.

(1946),."The nitration of dianisoylmethane and p-methoxydesoxybenzoin," *Chem. Ber.*, 29-46] mp 174°C).

1-Phenyl-3,5-di(4-methoxyphenyl)-pyrazole **(34b).** Diketone **33** (100 mg, 0.35 mmol) and phenyl hydrazine hydrochloride (500 mg, 3.5 mmol) were reacted as outlined in general pyrazole procedure above to afford **34b** [Ando, W., Sato, R., Yamashita, M., Akasaka, T. & Miyazaki, H. (1983), "Quenching of singlet oxygen by 1,3,5-triaryl-2-pyraazolines," *J. Org. Chem.* **48**, 542-546] (30 mg, 25%). mp 159-161°C (lit [Ando, W., Sato, R., Yamashita, M., Akasaka, T. & Miyazaki, H. (1983), "Quenching of singlet oxygen by 1,3,5-triaryl-2-pyraazolines," *J. Org. Chem.* **48**, 542-546] mp 163°C).

1-Benzyl-3,5-di(4-methoxyphenyl)-pyrazole **(34c).** Diketone **33** (300 mg, 1.07 mmol) and benzylhydrazine dihydrochloride (335 mg, 2.13 mmol) were reacted as outlined in the general pyrazole procedure above to afford **34c** (179 mg, 45%).

1,3,5-Tri(4-methoxyphenyl)-pyrazole **(34d).** Diketone **33** (100 mg, 0.35 mmol) and 4-methoxyphenyl hydrazine hydrochloride (92.2 mg, 0.53 mmol) were reacted as outlined in the general pyrazole procedure above to afford **34d** as a white solid (112 mg, 85%).

3,5-Di(4-hydroxyphenyl)-1H-pyrazole **(35a).** Pyrazole **34a** (20 mg, 0.07 mmol) was demethylated with BBr_3 according to the general procedure to afford **35a** [Hergenrother, P.M. (1991), "New Developments in Thermally Stable Polymers," *Rec. Travl. Chim. Pays-Bas.* **110**, 481-491] as an off-white solid (11 mg, 63%).

1-Phenyl-3,5-di(4-hydroxyphenyl)-pyrazole **(35b).** Pyrazole **34b** (20 mg, 0.06 mmol) was demethylated with BBr_3 according to the general procedure to afford **34b** [Hergenrother, P.M. (1991), "New Developments in Thermally Stable Polymers," *Rec. Travl. Chim. Pays-Bas.* **110**, 481-491] as an off-white solid (11.5 mg, 58%).

1-Benzyl-3,5-di(4-hydroxyphenyl)-pyrazole (**35c**). Pyrazole **34c** (178 mg, 0.48 mmol) was demethylated with BBr_3 according to the general procedure to afford **35c** as a yellow film (100 mg, 66%).

1,3,5-Tri(4-hydroxyphenyl)-pyrazole (**35d**). Pyrazole **34d** (112 mg, 0.29 mmol) was demethylated with BBr_3 according to the general procedure to afford **35d** (44.8 mg, 45%) as an off-white solid.

4-Ethyl-3,5-di(4-methoxyphenyl)-1H-pyrazole (**37a**). Diketone **36** (100 mg, 0.32 mmol) and hydrazine (0.12 mL, 3.2 mmol) were reacted as outlined in the general pyrazole procedure above to afford **37a** as a while solid (69 mg, 70%).

1-Phenyl-4-ethyl-3,5-di(4-methoxyphenyl)-pyrazole (37b). Diketone **36** (100 mg, 0.35 mmol) and phenyl hydrazine hydrochloride (140 mg, 0.96 mmol) were reacted as outlined in the general pyrazole procedure above to afford **37b** as an orange solid (109 mg, 87%).

1-Benzyl-4-ethyl-3,5-di(4-methoxyphenyl)-pyrazole (**37c**). Diketone **36** (200 mg, 0.64 mmol) and benzylhydrazine dihydrochloride (188.2 mg, 0.97 mmol) were reacted as outlined in the general pyrazole procedure above to afford **37c** as colorless film (80 mg, 31%).

4-Ethyl-1,3,5-tri(4-methoxyphenyl)-pyrazole (**37d**). Diketone **36** (50 mg, 0.16 mmol) and 4-methoxyphenyl hydrazine hydrochloride (140 mg, 0.96 mmol) were reacted as outlined in the general pyrazole procedure above to afford **37d** as an orange solid (11 mg, 23%).

4-Ethyl-3,5-di(4-hydroxyphenyl)-1H-pyrazole (**38a**). Pyrazole **37a** (69 mg, 0.22 mmol) was demethylated according to the general BBr_3 procedure to afford **38a** as a white solid (35 mg, 57%).

1-Phenyl-4-ethyl-3,5-di(4-hydroxyphenyl)-pyrazole (**38b**). Pyrazole **37b** (100 mg, 0.26 mmol) was demethylated according to the general BBr_3 procedure to afford **38b** as a white solid (50 mg, 54%).

1-Benzyl-4-ethyl-3,5-di(4-hydroxyphenyl)-pyrazole (**38c**). Pyrazole **37c** (80 mg, 0.20 mmol) was demethylated according to the general BBr_3 procedure to afford **38c** contaminated with a brominated side product. The two compounds were separated using RPHPLC (30:70, H_2O:MeOH, Partisil ODS2 C-18 prep column) (50 mg, 54%).

4-Ethyl-1,3,5-tri(4-hydroxyphenyl)-pyrazole (**38d**). Pyrazole **37d** (10 mg, 0.03 mmol) was demethylated according to the general BBr_3 procedure to afford **38d** (9.9 mg, 100%).

3,5-Di(4-methoxyphenyl)isoxazole (**40**). To a solution of oxime **39** (1.0 g, 6 mmol) in THF (20 mL) at 0°C was added nBuLi (9.11 mL, 13.3 mmol) as a solution in Hexanes. The clear solution was stirred for 30 min at 0°C then methyl 4-methoxybenzoate (498 mg, 3 mmol) was added as a solution in THF (5 mL) over 5 min. The reaction mixture was stirred at 0°C for 30 min, then warmed to room temperature. 5 N HCl (10 mL) was added and the biphasic reaction mixture was brought to reflux overnight (12 h). Upon cooling to 0°C, isoxazole **40** [Ichinose, N., Mizuno, K., Tami, T. & Otsuji, Y. (1988), "A novel NO insertion into cyclopropane ring by use of $NOBF_4$. Formation of 2-isoxazolines," *Chem. Lett.* , 233-236] precipitated and was collected via filtration (450 mg, 27%). mp 174-177°C (lit [Ichinose, N., Mizuno, K., Tami, T. & Otsuji, Y. (1988), "A novel NO insertion into cyclopropane ring by use of $NOBF_4$. Formation of 2-isoxazolines," *Chem. Lett.* , 233-236] mp 176-177°C);

3,5-Di(4-hydroxyphenyl)isoxazole (**41**). Isoxazole **40** (300 g, 1.1 mmol) was demethylated according to the general BBr_3 procedure to afford **41** [Murthy, A.K., Rao, K.S.R.K.M. & Rao, N.V.S. (1968) "Isoxazolylphenols and their absorption spectra," *Aus. J. Chem.* **21**, 2315-2317] as a white solid (152 mg, 56 %). mp 267-269°C (lit [Murthy, A.K., Rao,

K.S.R.K.M. & Rao, N.V.S. (1968) "Isoxazolylphenols and their absorption spectra," *Aus. J. Chem.* **21**, 2315-2317] mp 255°C);

General procedure for the synthesis of pyridazines:

A stirred solution of 1,4-dione (55.0 mg, 0.12 mmol) in hydrazine hydrate (5 mL) with a minimal amount of ethanol (to dissolve dione), was heated to reflux overnight. The reaction was allowed to cool to room temperature and then diluted with ethyl acetate (15 mL). The solution was transferred to a separatory and the aqueous layer washed with ethyl acetate. The organic extracts were pooled and washed with water, sat. sodium chloride, dried over sodium sulfate and filtered. Solvent was removed under reduced pressure to yield crude 4,5-dihydropyridazine. Flash column chromatography (1:4 EtOAc:hexanes) afforded pure 4,5-dihydropyridazine, which was taken up into methylene chloride and left exposed to air overnight. Any remaining methylene chloride was removed under reduced pressure to afford crude pyridazine. Purification by flash column chromatography (1:1 EtOAc:hexanes) followed by recrystallization (EtOAc:Hex) gave pure pyridazine.

Methoxy-protected pyridazine were deprotected according to the BF_3SMe_2 general demethylation procedure as described above to afford pyridazine analogs.

General procedure for the synthesis of pyrimidines:

To a well-stirred solution of the ketone (1 mmol) and the nitrile (2.2 mmol) in an. dichloroethane (5 ml) was slowly added triflic anhydride (1.1 mmol). The reaction mixture was stirred at room temperature for 24hr, satd. bicarbonate solution was added and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with brine and dried (an. Na_2SO_4). The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography over silica gel using 30% ethyl acetate-hexane as eluent to furnish the pyrimidines.

General procedure for the synthesis of pyrazines:

Method A (Scheme 13E): A magnetically stirred solution of the α–diketone (1mmol) and the diamine (1 mmol) in acetic acid (1.5 ml) was refluxed for 3.5-4.0 hr. The reaction mixture was cooled, poured into ice and extracted with ethyl acetate (3x5ml). The combined organic phases were washed with brine and dried (an. Na_2SO_4). Evaporation of the solvent and purification of the residue over a silica gel column using 20% ethyl acetate-hexane as eluent furnished the pyrazines.

Method B (Scheme 13F) To a stirred mixture of the α–hydroxy ketones (1mmol each) in ethanol (6 ml) was added ammonium acetate (3 mmol). The reaction mixture was refluxed for 4hr, cooled and poured into ice, the precipitated solid filtered off and was washed with cold water. The residue was purified over a silica gel column using 20% ethyl acetate-hexane as eluent to furnish the mixture of pyrazines.

General protocol for deprotection of phenolic methyl ethers:

To a stirred solution of the protected pyrimidine (1 mmol) or pyrazine (1 mmol) in dichloromethane was added boron triflouride-dimethylsulfide complex (10 mmol/phenolic gp.) and the reaction stirred at room temperature for 24-36 hr. After quenching with water, the layers were separated and the aqueous layer extracted with ethyl acetate (3x10 ml). The combined extracts were washed with satd. bicarbonate solution, brine and dried (an. Na_2SO_4). Evaporation of the solvent and purification of the residue over a silica gel column using 50% ethyl acetate-hexane or ethyl acetate as eluent furnished the free phenolic pyrimidine or pyrazine.

General experimental procedure for synthesis of quinoxalines:

Step 1: A well stirred mixture of the *o*-phenylenediamine dihydrochloride (1 mmol) and the α-diketone (1 mmol) in acetic acid were refuxed for 3.5-4.0 hr. The reaction mixture was cooled, poured into ice and extracted with ethyl acetate (3x10 ml). The combined extracts were washed with brine, dried (an. Na_2SO_4) and concentrated. Purification of the residue over a silica gel column using 30% ethyl acetate-hexane as eluent furnished an ~1:1 unseparable mixture of the quinoxalines.

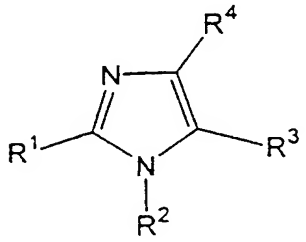
Step 2: To a magnetically stirred solution of the isomeric mixture of protected quinoxalines (1mmol) in dichloromethane was added boron triflouride-dimethyl sulfide (10 mmol/phenolic gp.) and the stirring continued for 2 days at room temperature. After quenching with water, the layers were separated and the aqueous layer extracted with ethyl acetate (3x10 ml). The combined extracts were washed with satd. bicarbonate solution, brine and dried (an. Na_2SO_4). Evaporation of the solvent and purification of the residue over a silica gel column using ethyl acetate as eluent furnished the deprotected quinoxalines.

Those of ordinary skill in the art will appreciate that starting materials, reagents, reaction conditions, methods, techniques, purification and isolation methods other those specifically detailed herein can be employed or readily adapted in view of well-know principles to make and use the compounds of this invention. All art-known equivalents of starting materials, reagents, reaction conditions, methods, techniques, purification and isolation methods described herein are intended to be encompassed by this invention.

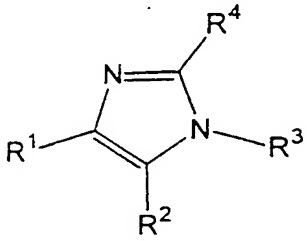
All references cited herein are incorporated in their entirety herein to the extent that they are not inconsistent with the disclosure herein

TABLE 1: EXEMPLARY STRUCTURES OF FIVE-MEMBERED RING CORES

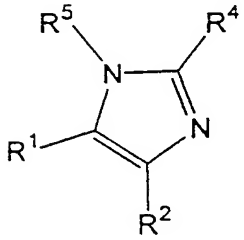
IMIDAZOLES



IM1



IM2



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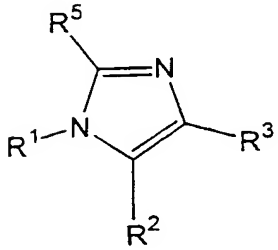
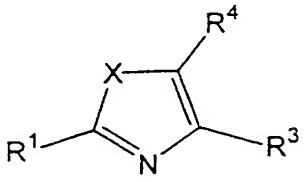


IM4

OXAZOLES/THIAZOLES



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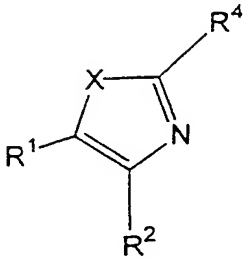

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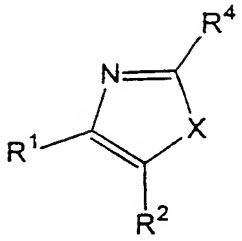
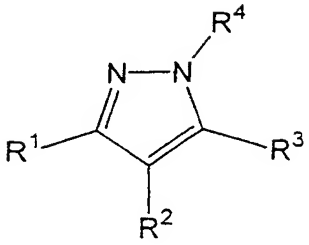


X = O, OA3
X = S, TA3

Table 1 (continued)

PYRAZOLES



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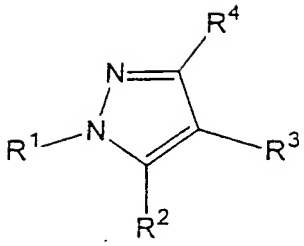

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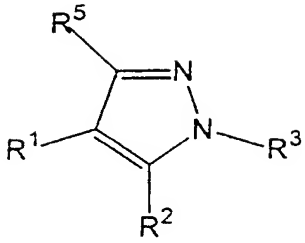
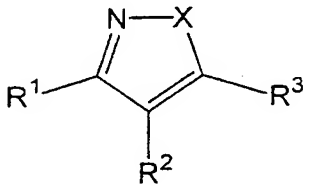


PA3

ISOXAZOLE/ISOTHIAZOLE



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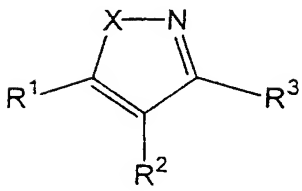

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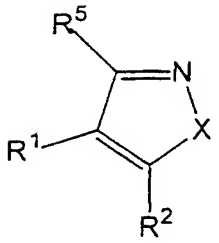
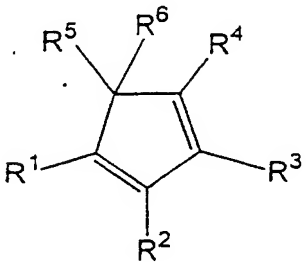
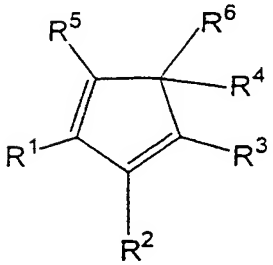


X = O, IO3
X = S, IS3

CYCLOPENTADIENES



C1



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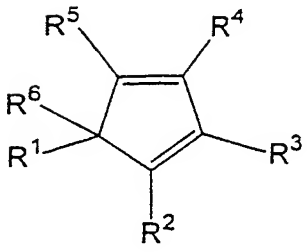
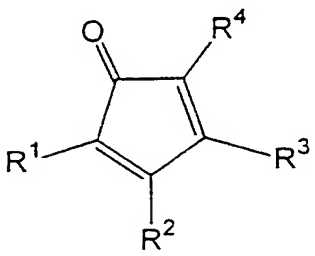


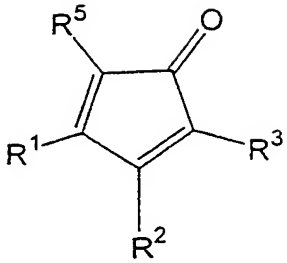
C3

Table 1(continued)

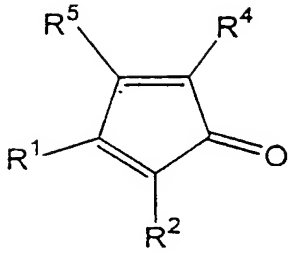
CYCLOPENTADIENEONES



CD1

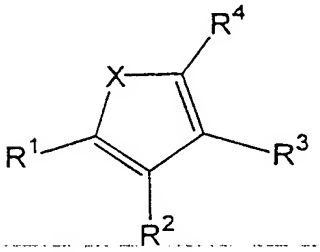


CD2



CD3

FURANS/THIOPHENES



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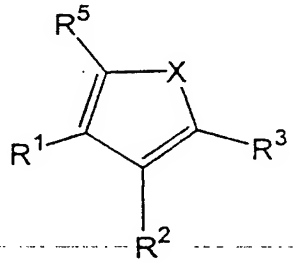
X = S, T1



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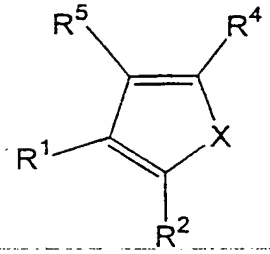

X = O, F3
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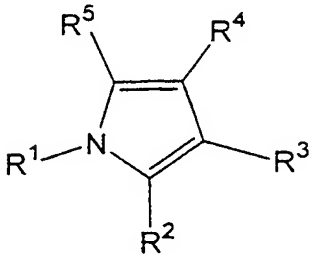
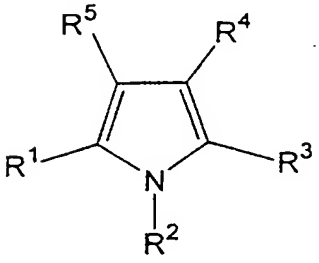
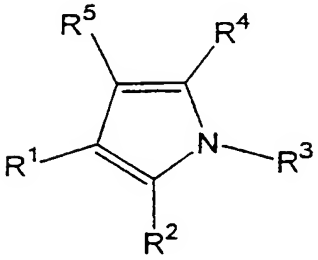
PYRROLES



PR1



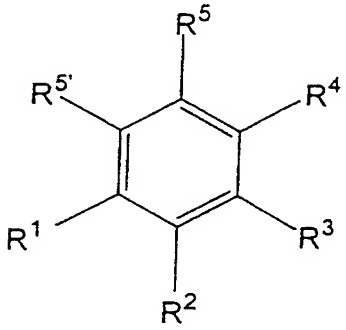
PR2



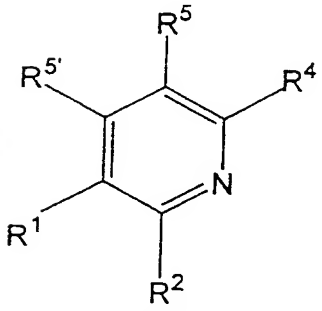
PR3

TABLE 2: EXEMPLARY STRUCTURES OF SIX-MEMBERED RING CORES

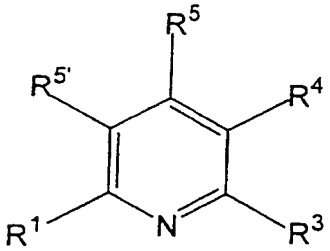
BENZENES



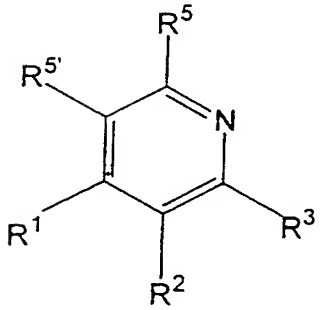
PYRIDINES



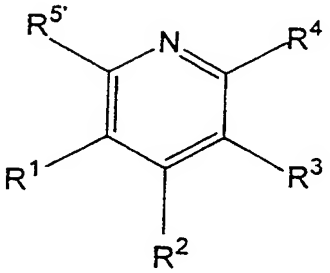
PY1



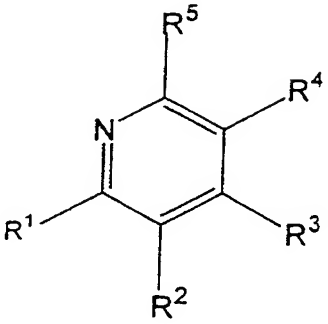
PY2



PY3



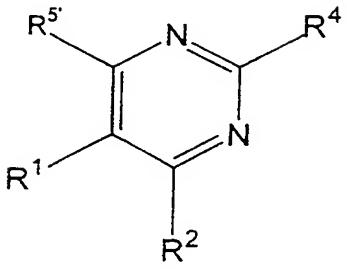
PY4



PY5

Table 2 (Continued)

PYRIMIDINES



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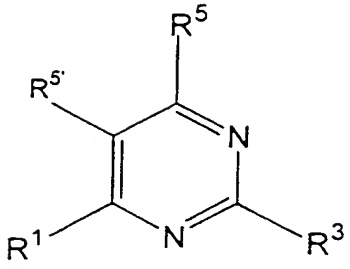

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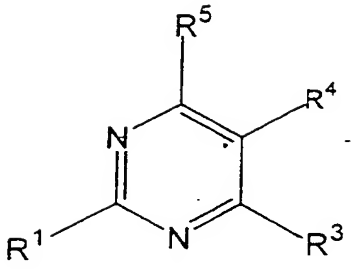
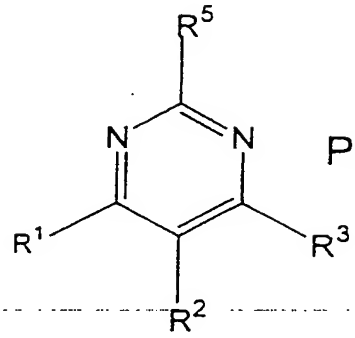
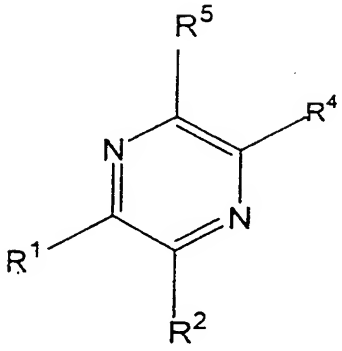


PM3



PM4

PYRAZINES



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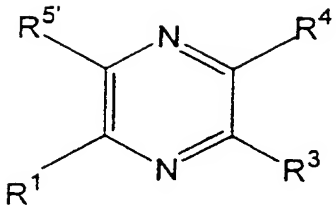

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Table 2(Continued)

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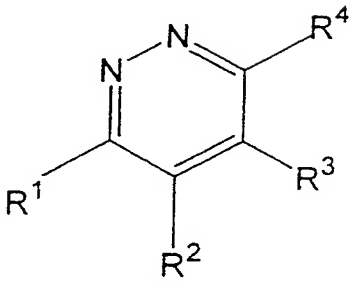

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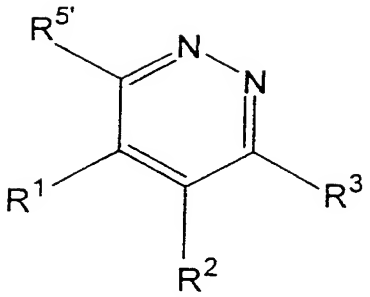
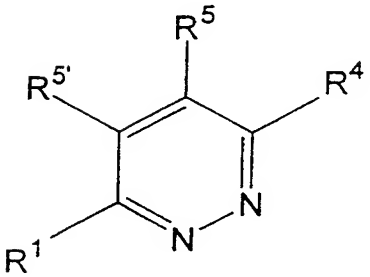
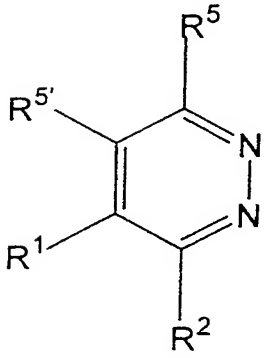


PZD2



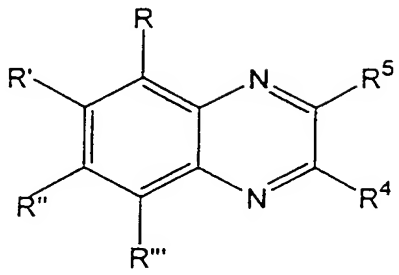
PZD3



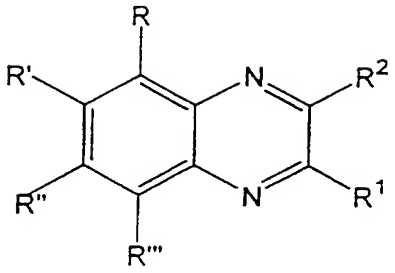
PZD4

Table 2 (Continued)

Quinoxalines

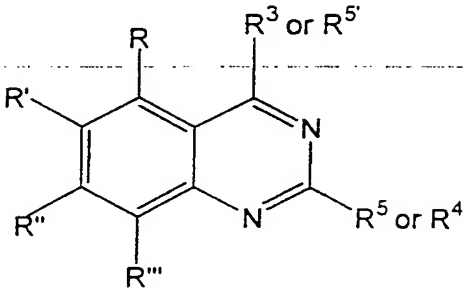


QX1

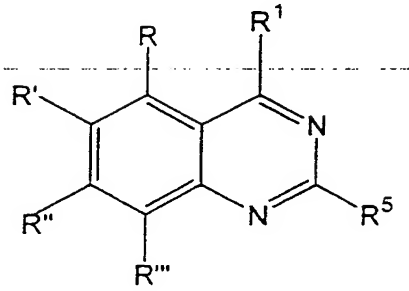


QX2

Quinazolines



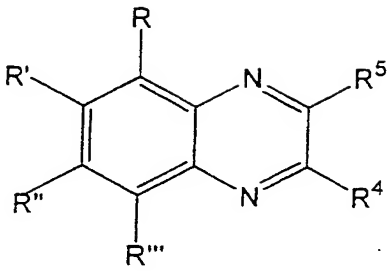
QZ1



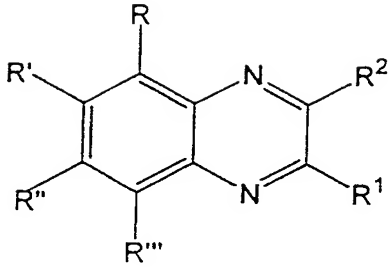
QZ2

Table 2(Continued)

Cinnolines

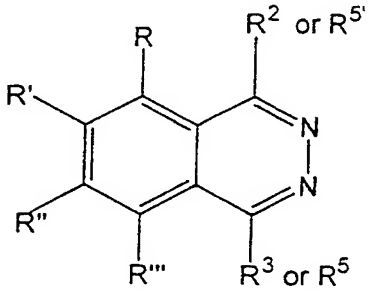


CN1

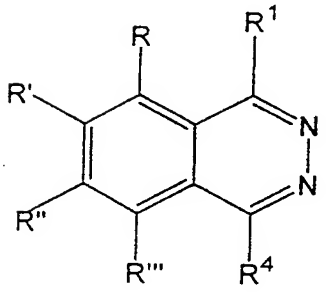


CN2

Phthalazines

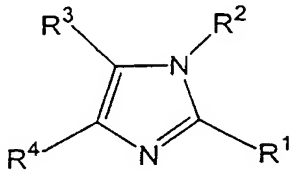


PH1



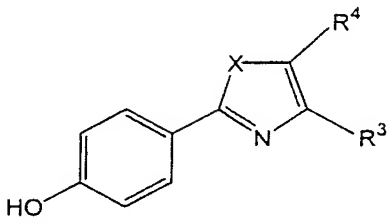
PH2

Table 3: ESTROGEN RECEPTOR BINDING DATA FOR IMIDAZOLES 3, 6A-D, 12 AND 17



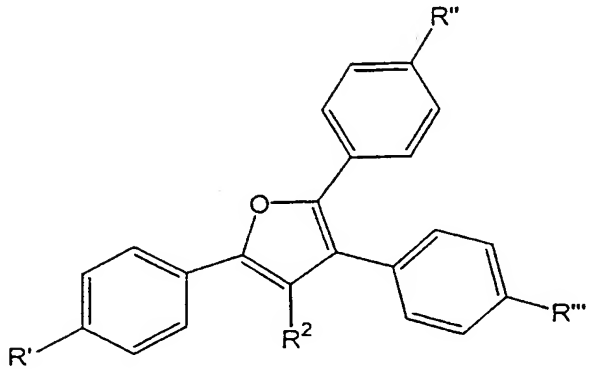
Compound	R^4	R^3	R^2	R^1	RBA
3	4'-HO-C_6H_4	4'-HO-C_6H_4	H	H	<0.001
6a	4'-HO-C_6H_4	4'-HO-C_6H_4	H	4'-HO-C_6H_4	0.007
6b	4'-HO-C_6H_4	4'-HO-C_6H_4	C_2H_5	4'-HO-C_6H_4	0.38
6c	4'-HO-C_6H_4	4'-HO-C_6H_4	C_3H_7	4'-HO-C_6H_4	0.62
6d	4'-HO-C_6H_4	4'-HO-C_6H_4	C_4H_9	4'-HO-C_6H_4	0.17
12	C_6H_5	4'-HO-C_6H_4	C_2H_5	4'-HO-C_6H_4	0.25
17	4'-HO-C_6H_4	C_2H_5	4'-HO-C_6H_4	C_6H_5	0.37

Table 4: ESTROGEN RECEPTOR BINDING DATA FOR THIAZOLES 22AB AND OXAZOLES 29 AND 31



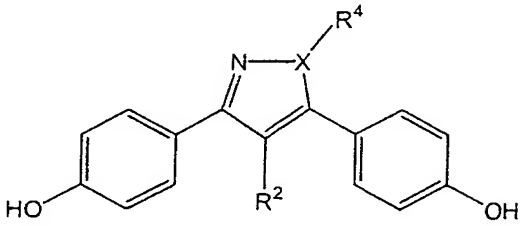
Compound	X	R^3	R^4	RBA
22a	S	4′-HO-C_6H_4	H	0.018
22b	S	4′-HO-C_6H_4	C_2H_5	0.041
29	O	4′-HO-C_6H_4	C_6H_5	<0.001
31	O	C_6H_5	4′-HO-C_6H_4	0.027

Table 5: ER BINDING AFFINITIES FOR EXEMPLARY FURANS



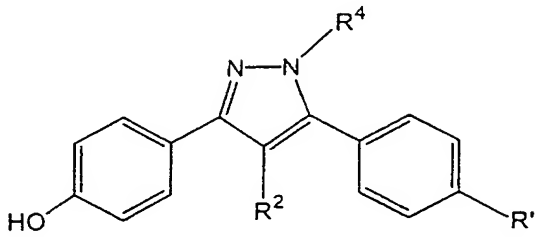
	R^2	R′	R″	R‴	BA(%)	ERα(%)	ERβ(%)
201	C_2H_5	OH	H	OH	5.01	67.6	6.31
202	n-C_3H_7	OH	H	OH	3.89		
203	C_2H_5	OH	OH	OH	5.89	214	3.02
204	n-C_3H_7	OH	OH	OH	9.33	85.1	2.4
	H	OH	H	OH	0.04		
	H	OH	OH	H	0.13		
200	C_2H_5	OH	OH	H	0.71	15.1	2.51

Table 6A: ESTROGEN RECEPTOR BINDING AFFINITY DATA FOR PYRAZOLES AND ISOXAZOLE



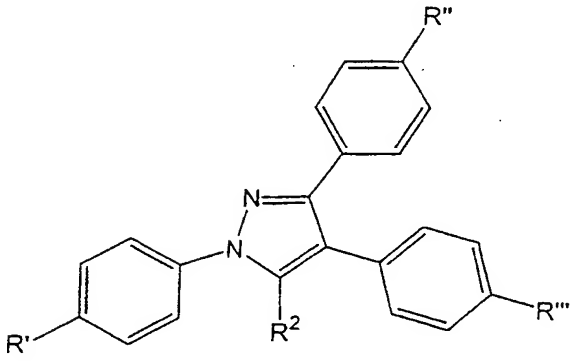
Compound	X	R^4	R^2	RBA
35a	N	H	H	0.009
35b	N	C_6H_5-	H	0.028
35c	N	$C_6H_5CH_2$-	H	<0.007
35d	N	$pHOC_6H_4$-	H	0.059
38a	N	H	C_2H_5	0.015
38b	N	C_6H_5-	C_2H_5	14.0
38c	N	$C_6H_5CH_2$-	C_2H_5	0.150
38d	N	$pHOC_6H_4$-	C_2H_5	19.0
41	O	--	C_2H_5	0.006

Table 6B: ESTROGEN RECEPTOR BINDING AFFINITIES REPRESENTATIVE FOR PYRAZOLES



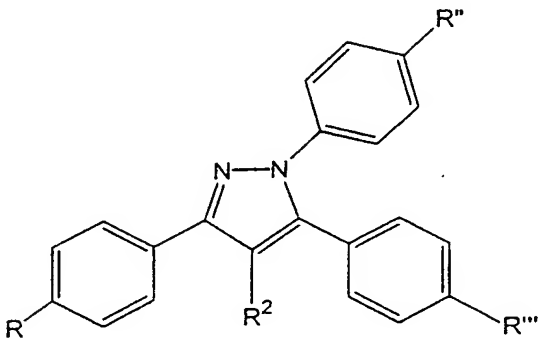
R_2	R'	R_4	%RBA
H	OH	H	0.009
H	OH	C_6H_5	0.028
H	OH	$C_6H_5CH_2$	<0.007
H	OH	p-HOC_6H_4	0.059
C_2H_5	OH	H	0.015
C_2H_5	OH	C_6H_5	14
C_2H_5	OH	$C_6H_5CH_2$	0.47
C_2H_5	OH	p-HOC_6H_4	20
CH_3	OH	C_6H_5	1.6
C_2H_5	OH	CH_2CH_2OH	1.2
C_3H_7	OH	C_6H_5	25

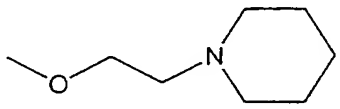
Table 7: ER BINDING AFFINITIES FOR EXEMPLARY PYRAZOLE ISOMERS



	R′	R″	R‴	R^2	RBA(%)
210	H	H	OH	C_2H_5	0.008 ± 0.005
211	OH	H	H	C_2H_5	0.43 ± 0.07
212	OH	H	OH	C_2H_5	5.6
213	OH	H	OH	n-C_3H_7	15
214	OH	OH	OH	C_2H_5	13

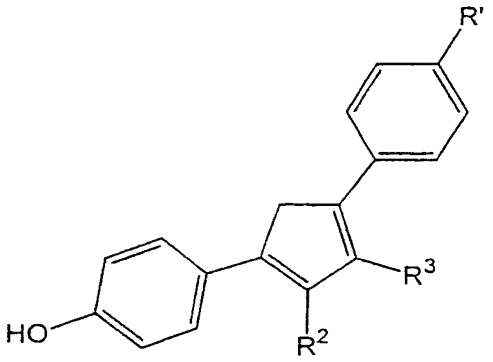
Table 8: ER BINDING AFFINITIES FOR PYRAZOLES WITH BASIC SIDE GROUPS.



PI = 

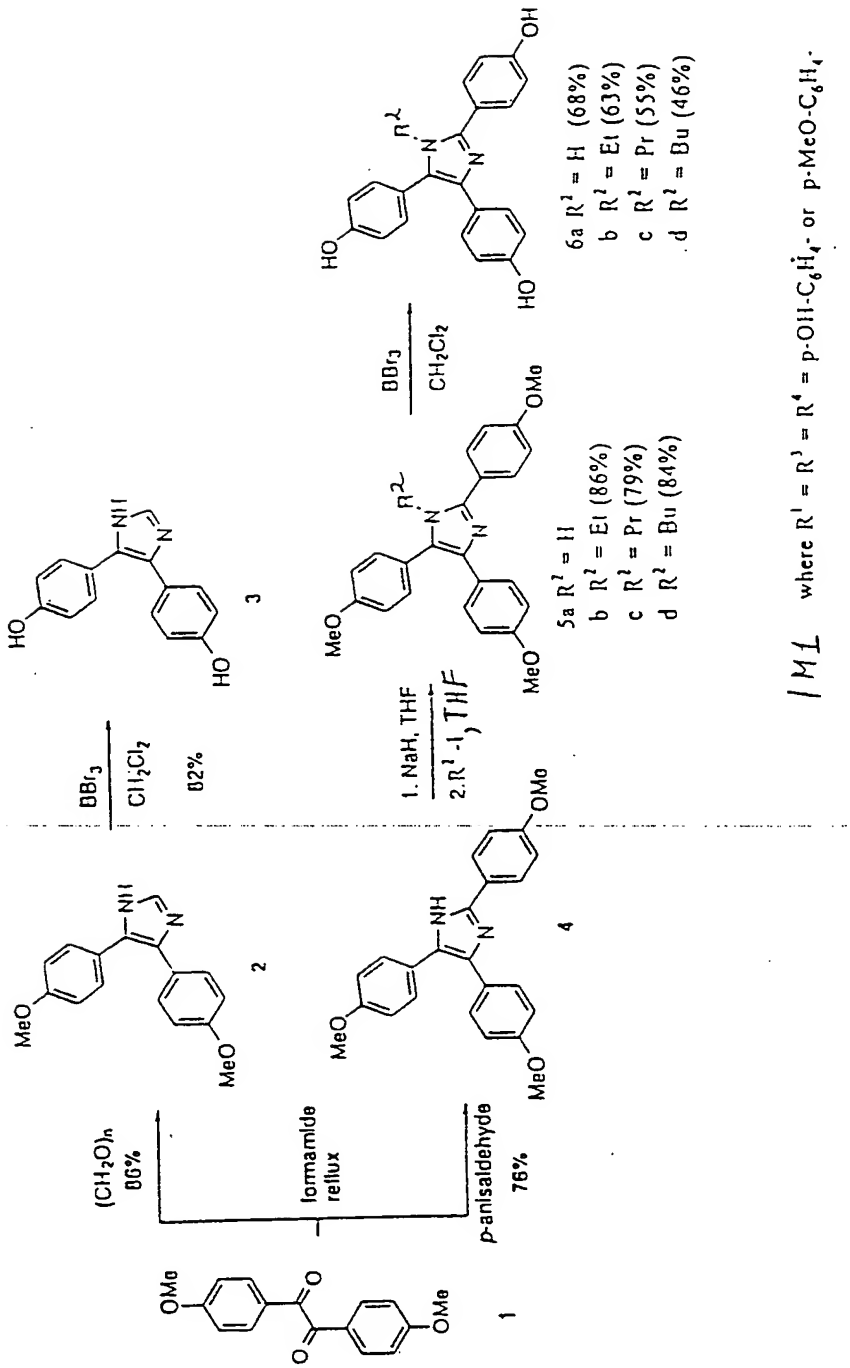
	R	R"	R'''	R^2	RBA% cytosol	$RBA_{ER\alpha}$	$RBA_{ER\beta}$
	OH	PI	OH	C_2H_5	2.0		
	OH	H	OH	PI	0.013		
	PI	OH	OH	C_2H_5	0.40		
301	OH	OH	PI	C_2H_5	32 ± 6.4	5.1 ± 1.6	0.18 ± 0.17

Table 9: RELATIVE ER BINDING AFFINITY DATA FOR CYCLOPENTADIENES

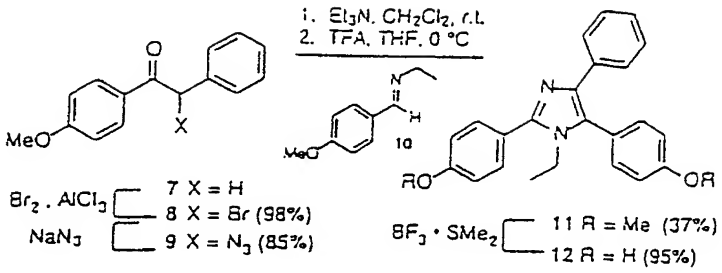


	R′	R^2	R^3	RBA (%)	RBA ERα	RBA ERβ
	H	C_2H_5	C_2H_5	0.028	0.372	0.355
	OH	C_2H_5	C_2H_5	1.047		
	H	C_2H_5	C_6H_5	0.05	1.26	0.50
	OH	C_2H_5	C_6H_5	1.20	8.32	7.08
235	OH	C_2H_5	p-OH-C_6H_5	8.91	5.25	1.66
	OH	n-C_3H_7	C_6H_5	0.06	0.33	0.708
	OH	n-C_3H_7	p-OH-C_6H_5	1.12	8.51	1.66

Scheme 1A

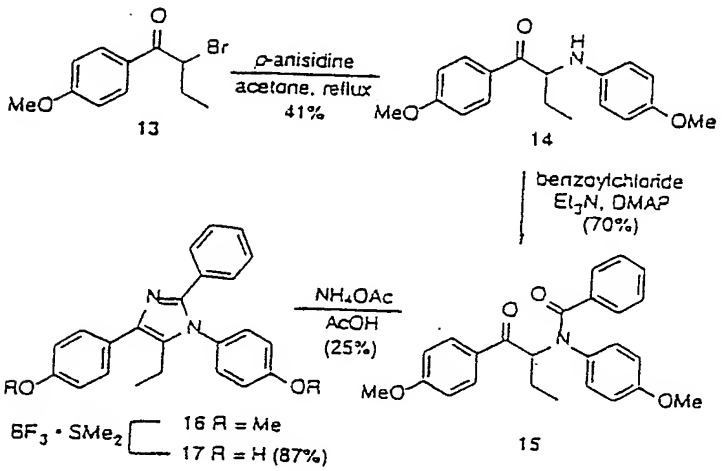


Scheme 1B



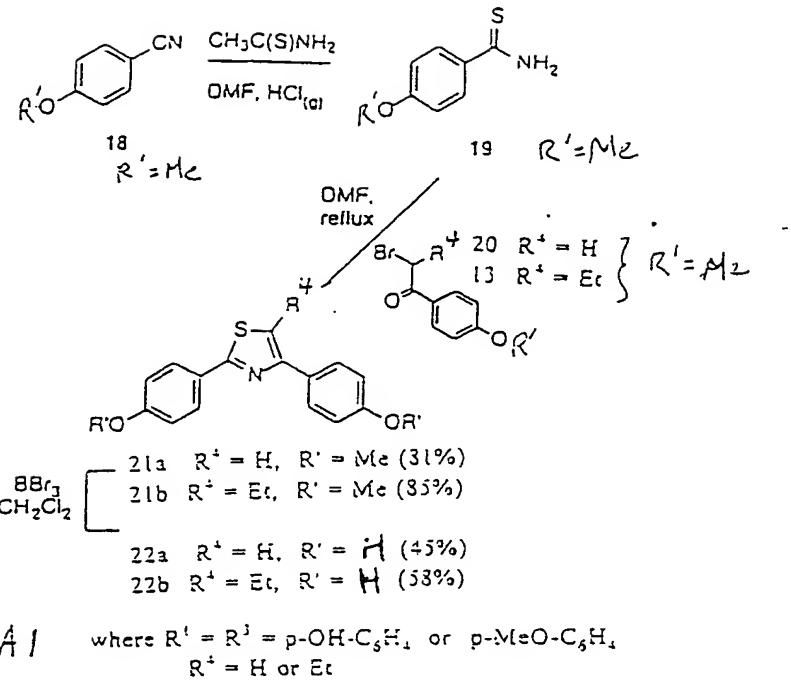
IM1 where $R^1 = R^3 =$ p-OH-C_6H_4 or p-MeO-C_6H_4
$R^4 = C_6H_5$
$R^2 =$ Et

Scheme 2



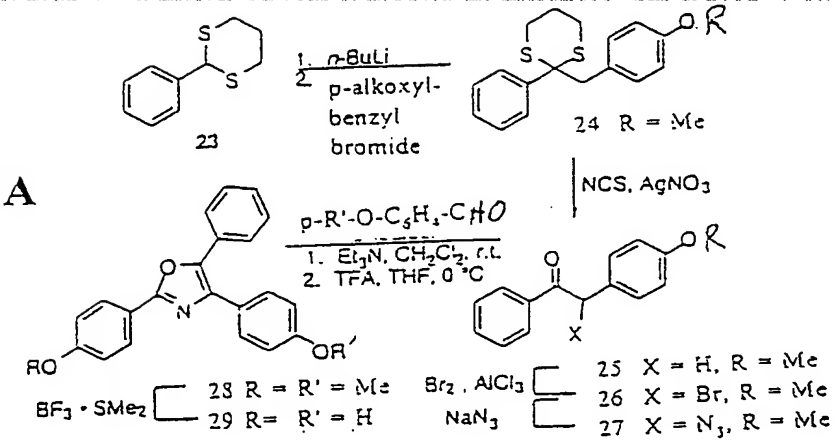
IM2 where $R^1 = R^3 =$ p-OH-C_6H_4 or p-MeO-C_6H_4
$R^4 = C_6H_5$-
$R^2 =$ Et

Scheme 3



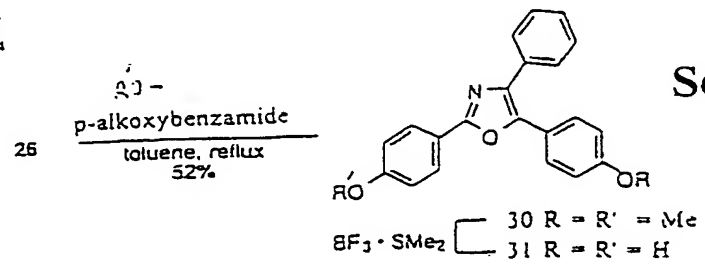
TA1 where $R^1 = R^3 = p\text{-}OH\text{-}C_6H_4$ or $p\text{-}MeO\text{-}C_6H_4$
$R^4 = H$ or Et

Scheme 4A

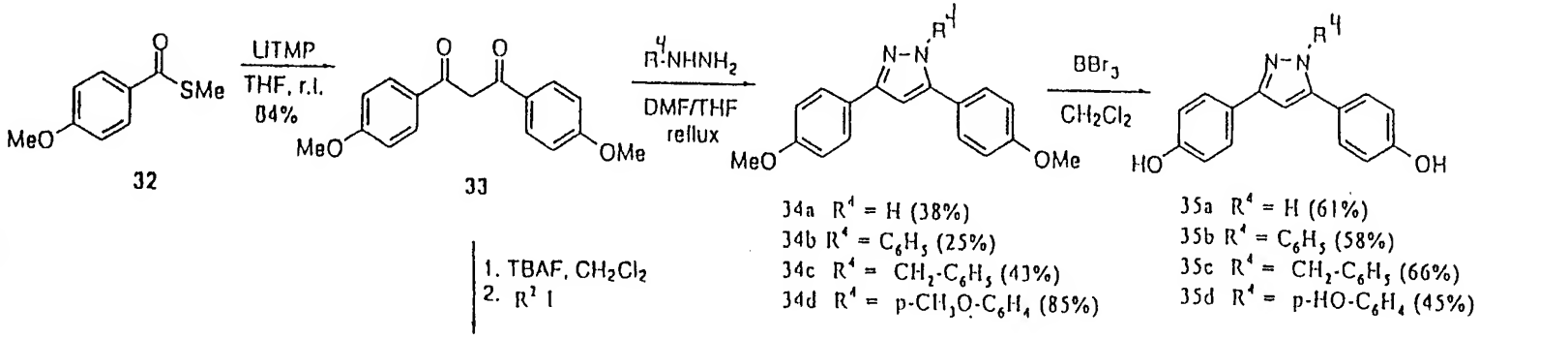


OA1 R^1 = p-R-O-C_6H_4
R^3 = p-R'-O-C_6H_4
$R^4 = C_6H_5$

Scheme 4B



Scheme 5A



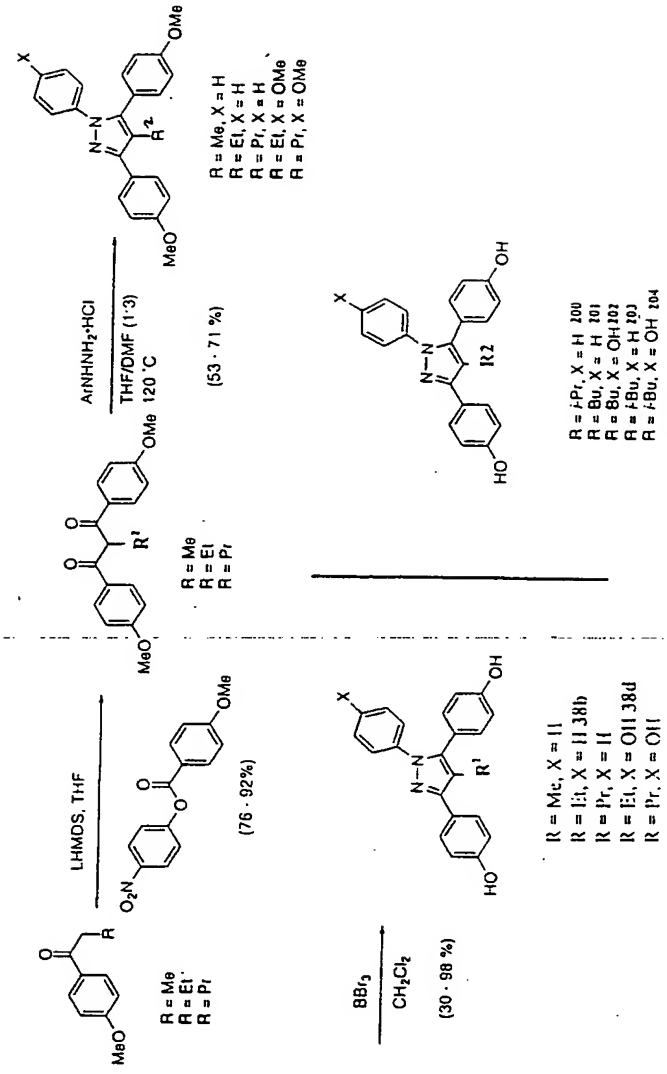
34a R^4 = H (38%)
34b R^4 = C_6H_5 (25%)
34c R^4 = CH_2-C_6H_5 (43%)
34d R^4 = p-CH_3O-C_6H_4 (85%)

35a R^4 = H (61%)
35b R^4 = C_6H_5 (58%)
35c R^4 = CH_2-C_6H_5 (66%)
35d R^4 = p-HO-C_6H_4 (45%)

37a R^4 = H, R^2 = Et (70%)
37b R^4 = C_6H_5, R^2 = Et (89%)
37c R^4 = CH_2-C_6H_5, R^2 = Et (31%)
37d R^4 = p-CH_3O-C_6H_4, R^2 = Et (23%)

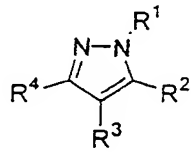
38a R^4 = H, R^2 = Et(57%)
38b R^4 = C_6H_5, R^2 = Et (54%)
38c R^4 = CH_2-C_6H_5, R^2 = Et (54%)
38d R^4 = p-HO-C_6H_4, R^2 = Et (99%)

PA1

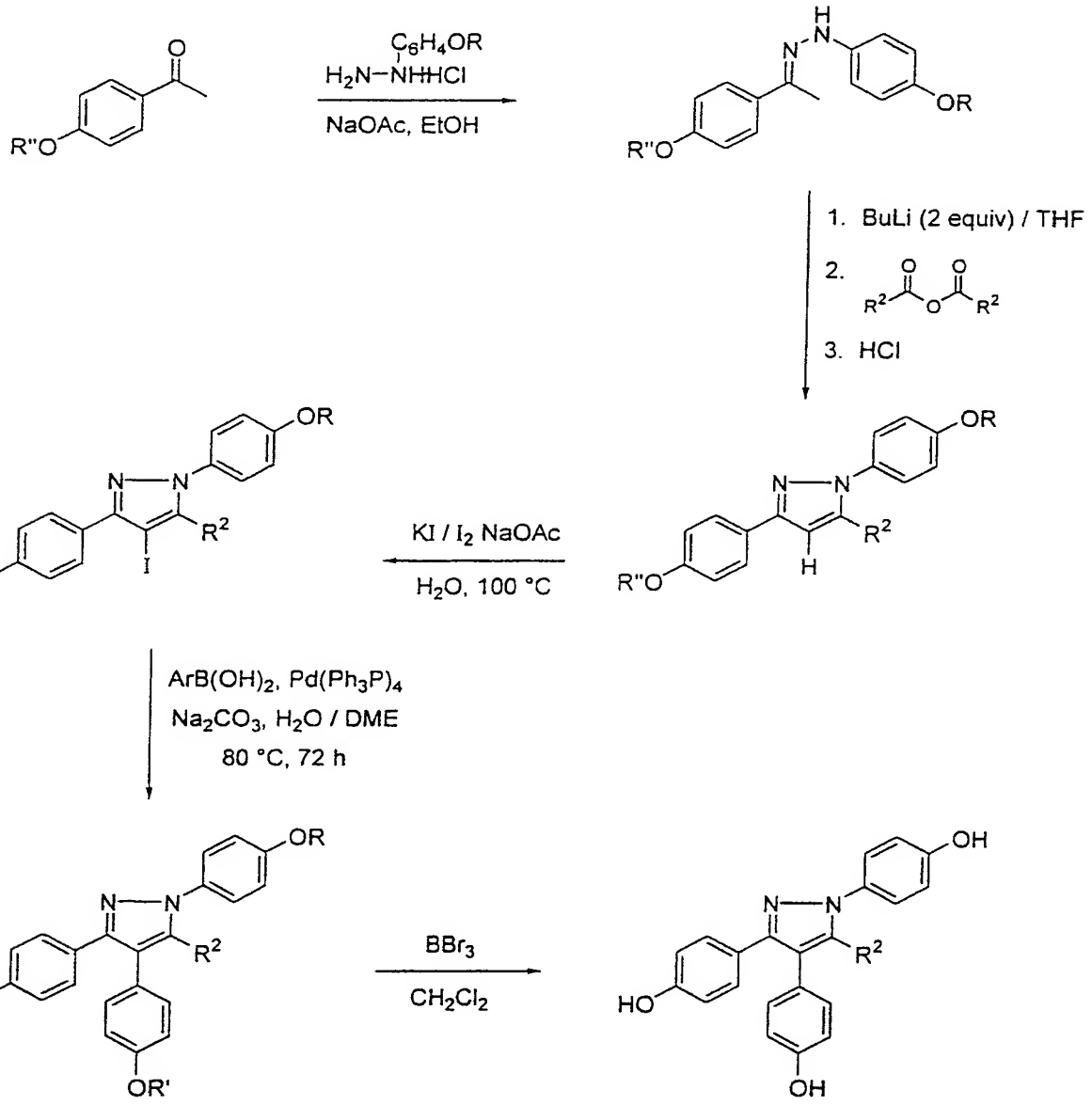


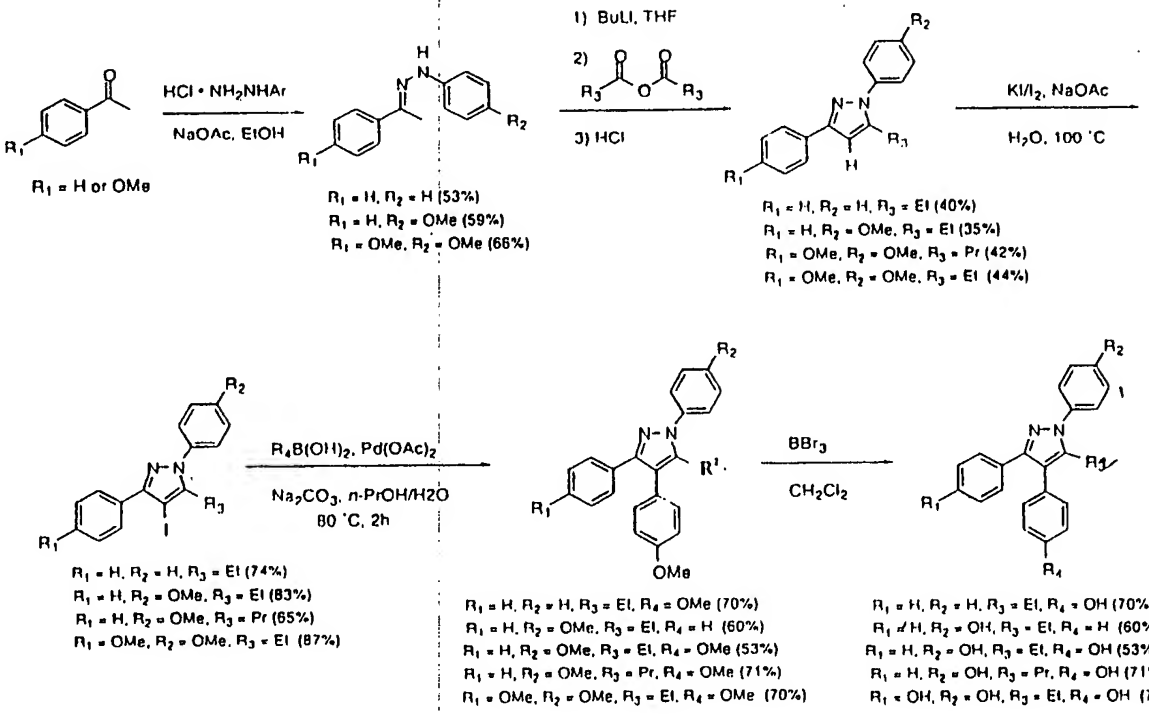
Scheme 5B Synthesis of C(4) alkyl pyrazole analogs. [this scheme is here and visible in "page layout", but not in "normal" view? It also prints fine.]

Scheme 5C

For synthesis of: PA2 

particularly: $R^1 = C_6H_4\text{-}OR$, $R = CH_3$, H
$R^3 = X$, $C_6H_4\text{-}OR'$, $R' = CH_3$, H
$R^4 = C_6H_5$, $C_6H_4\text{-}OR''$, $R'' = CH_3$, H

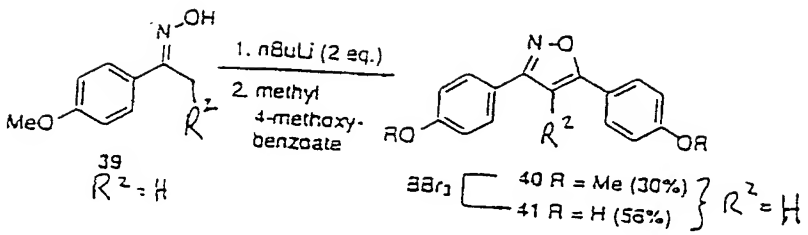




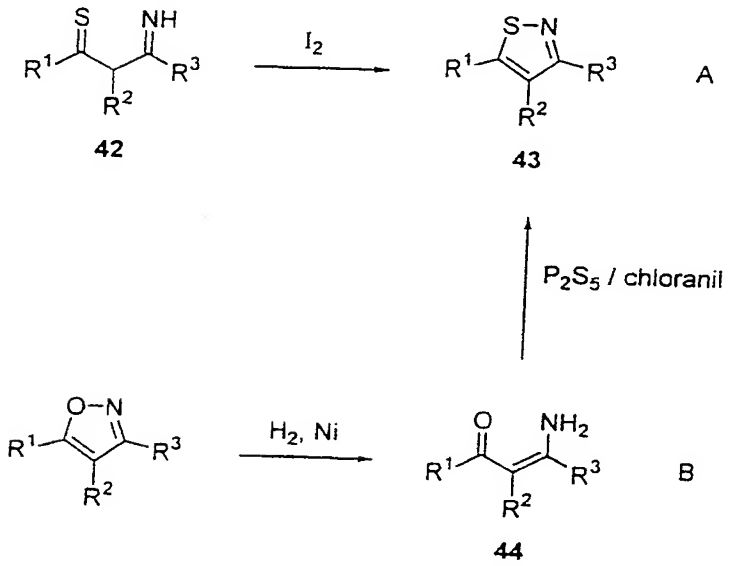
Scheme 5D Synthesis of 1,3,4-triaryl-5-alkylpyrazoles.

PCT/US99/22747

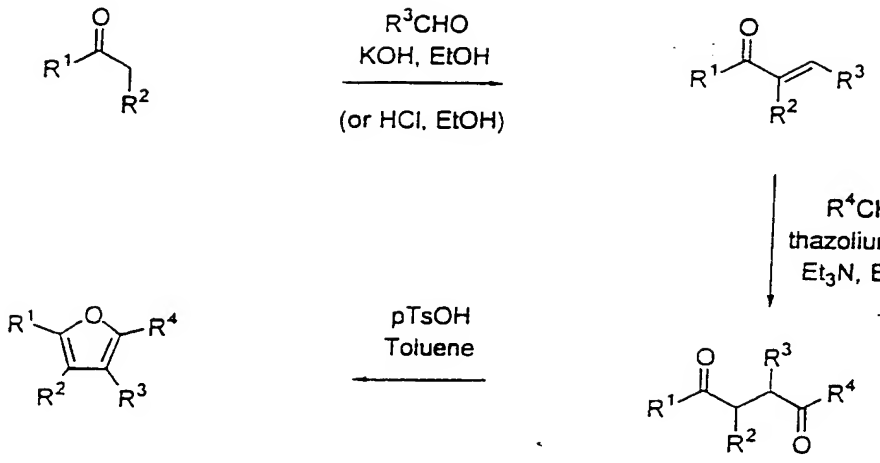
Scheme 6



Scheme 7



Scheme 8



57 $R^1 = C_6H_5$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = C_2H_5$
58 $R^1 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = p\text{-}CH_3O\text{-}C_6H_4$
59 $R^1 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = C_6H_5$

53 $R^1 = C_6H_5$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = C_2H_5$
54 $R^1 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = p\text{-}CH_3O\text{-}C_6H_4$
56 $R^1 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = C_6H_5$

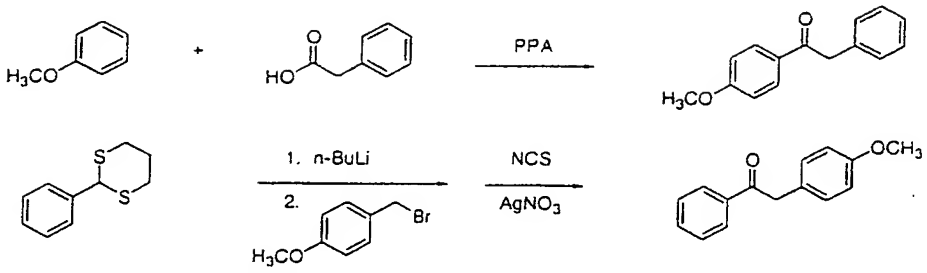
(if $R^2 = H$)
Br_2, CS_2 (I)
or I_2 (II)
or NCS (III)

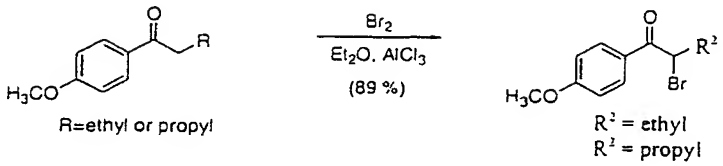
$BF_3\text{-}SMe_2$
CH_2Cl_2

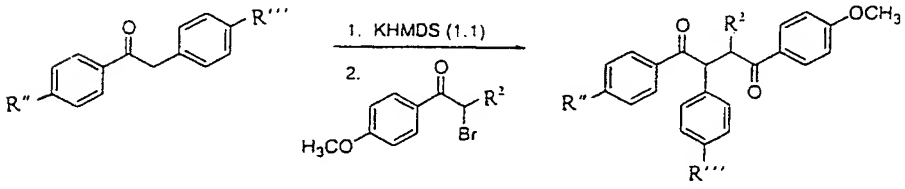
$R^2 = Y$
if I, then Y = Br
if II, then Y = I
if III, then Y = Cl

$R^1 = C_6H_5$, $R^2 = H$, $R^3 = HO\text{-}C_6H_4$, $R^4 = C_2H_5$
$R^1 = HO\text{-}C_6H_4$, $R^2 = H$, $R^3 = HO\text{-}C_6H_4$, $R^4 = HO\text{-}C_6H_4$
$R^1 = HO\text{-}C_6H_4$, $R^2 = H$, $R^3 = HO\text{-}C_6H_4$, $R^4 = C_6H_5$

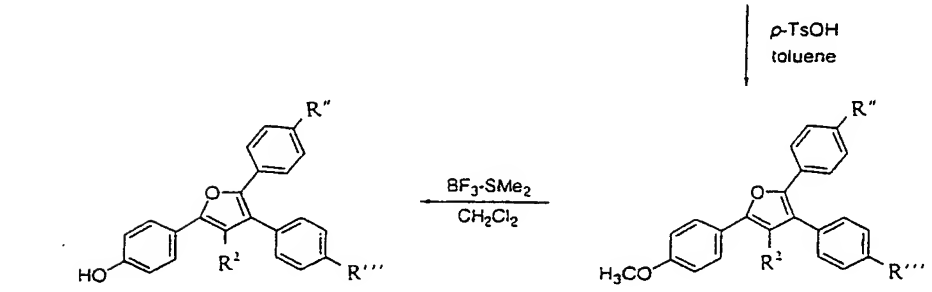
Scheme 8A







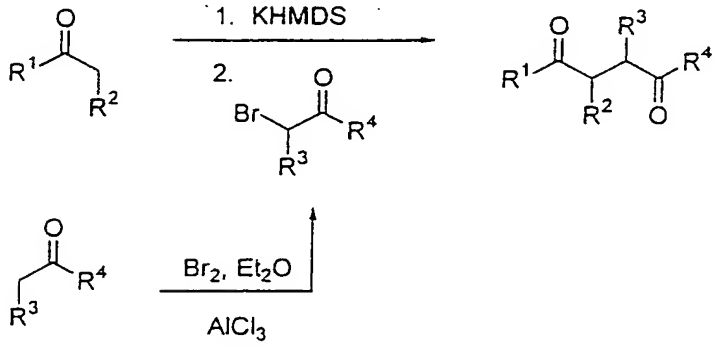
R^1= OCH_3, R^2= H, R^3= ethyl
R^1= H, R^2= OCH_3, R^3= ethyl
R^1= H, R^2= OCH_3, R^3= propyl
R^1=R^2= OCH_3, R^3= ethyl
R^1=R^2= OCH_3, R^3= propyl



R^1= OH, R^2= H, R^3= ethyl 210
R^1= H, R^2= OH, R^3= ethyl 211
R^1= H, R^2= OH, R^3= propyl 212
R^1= R^2= OH, R^3= ethyl 213
R^1= R^2= OH, R^3= propyl 214

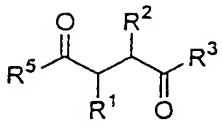
R"= OCH_3, R'''= H, R^2= ethyl
R"= H, R'''= OCH_3, R^2= ethyl
R"= H, R'''= OCH_3, R^2= propyl
R"= R"= OCH_3, R^2= ethyl
R"= R'''= OCH_3, R^2= propyl

Scheme 8B alternative 1,4-dione synthesis, particularly: $R^1 = C_6H_5$, C_6H_4-OR, R = CH_3, H
$R^2 = C_6H_5$, C_6H_4-OR', R' = CH_3, H
$R^4 = C_6H_5$, C_6H_4-OR", R" = CH_3, H



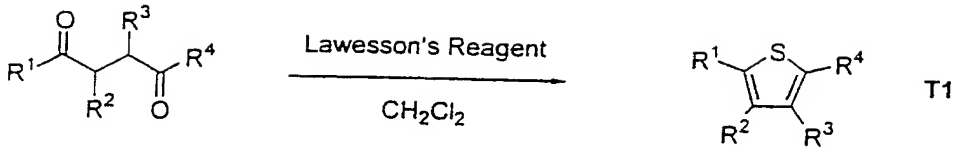
Can also be used to make:

alternative 1,4-dione synthesis, particularly:



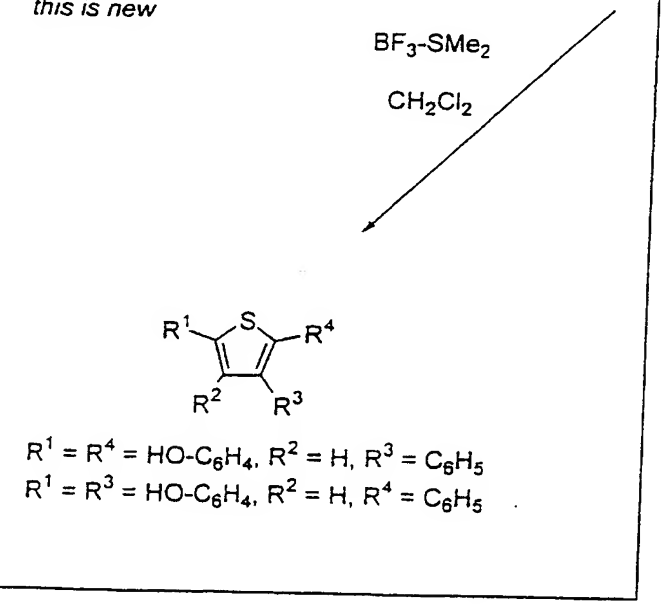
$R^1 = C_6H_5$, C_6H_4-OR, R = CH_3, H
$R^3 = C_6H_5$, C_6H_4-OR', R' = CH_3, H
$R^5 = C_6H_5$, C_6H_4-OR", R" = CH_3, H

Scheme 9



60 $R^1 = R^4 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^3 = C_6H_5$
61 $R^1 = R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^4 = C_6H_5$

this is new

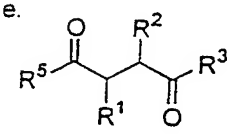


$R^1 = R^4 = HO\text{-}C_6H_4$, $R^2 = H$, $R^3 = C_6H_5$
$R^1 = R^3 = HO\text{-}C_6H_4$, $R^2 = H$, $R^4 = C_6H_5$

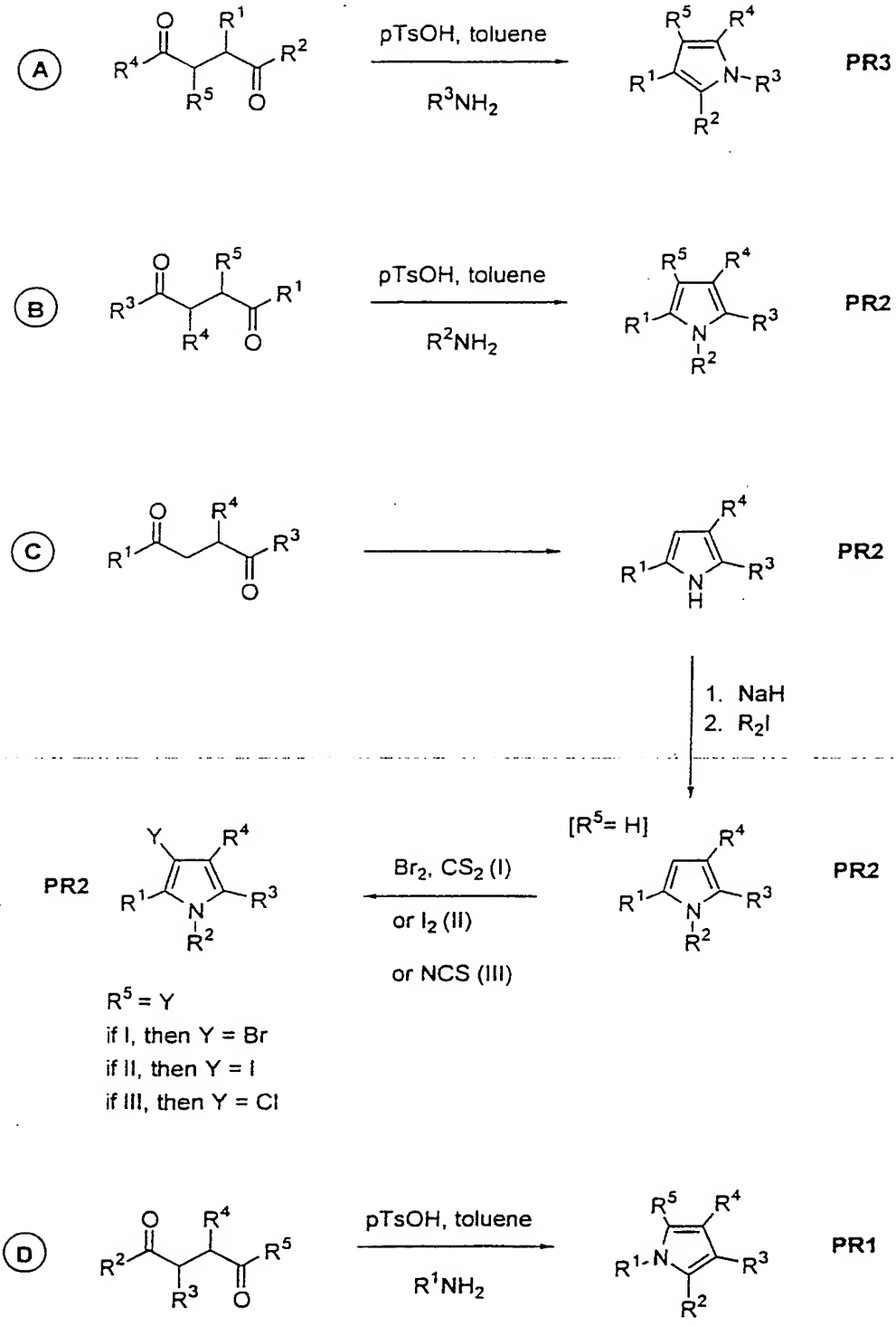
(if $R^2 = H$)
Br_2, CS_2 (I)
or I_2 (II)
or NCS (III)

$R^2 = Y$
if I, then Y = Br
if II, then Y = I
if III, then Y = Cl

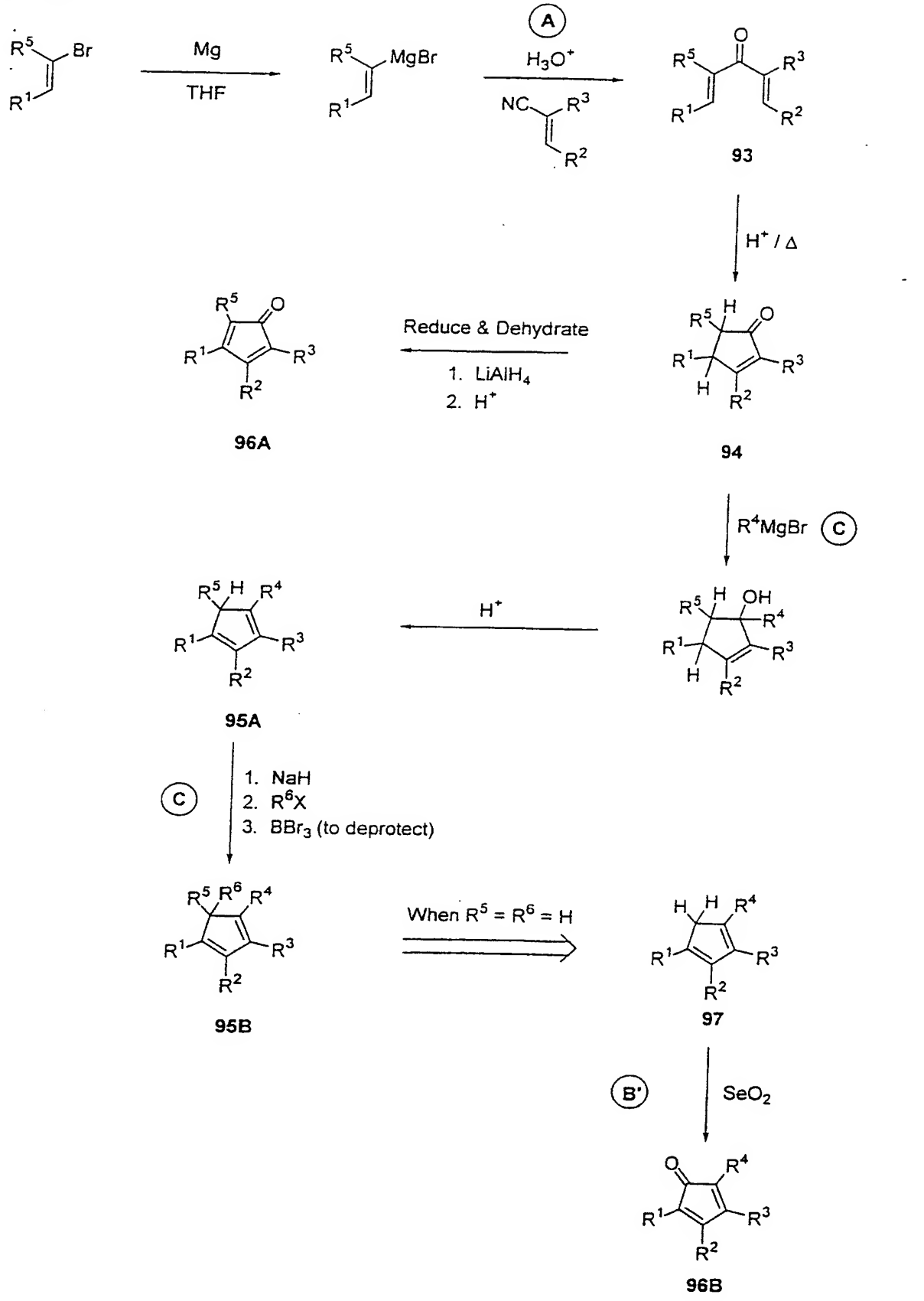
T2 Synthesized by choice of starting diketone i.e.



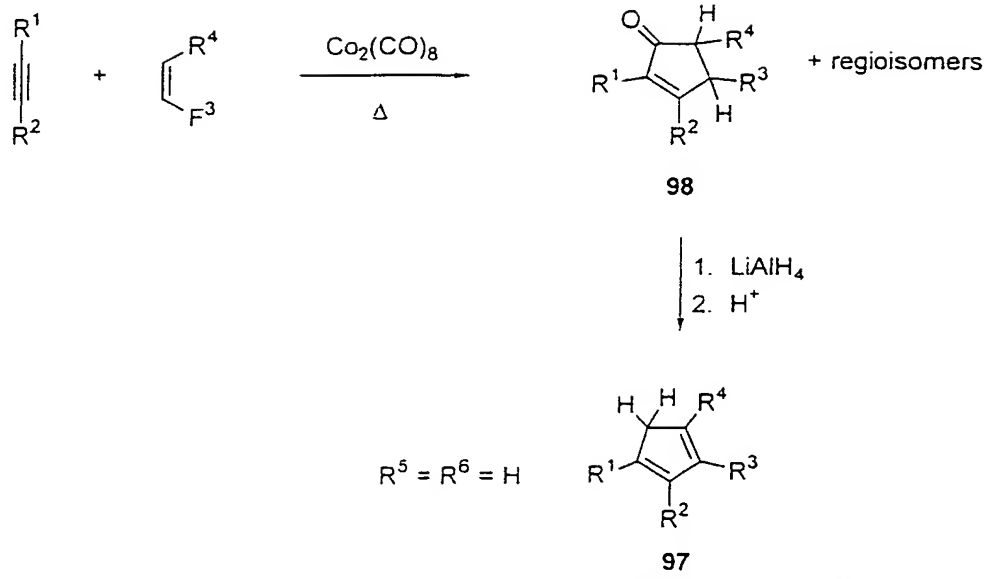
Schemes 10A-D



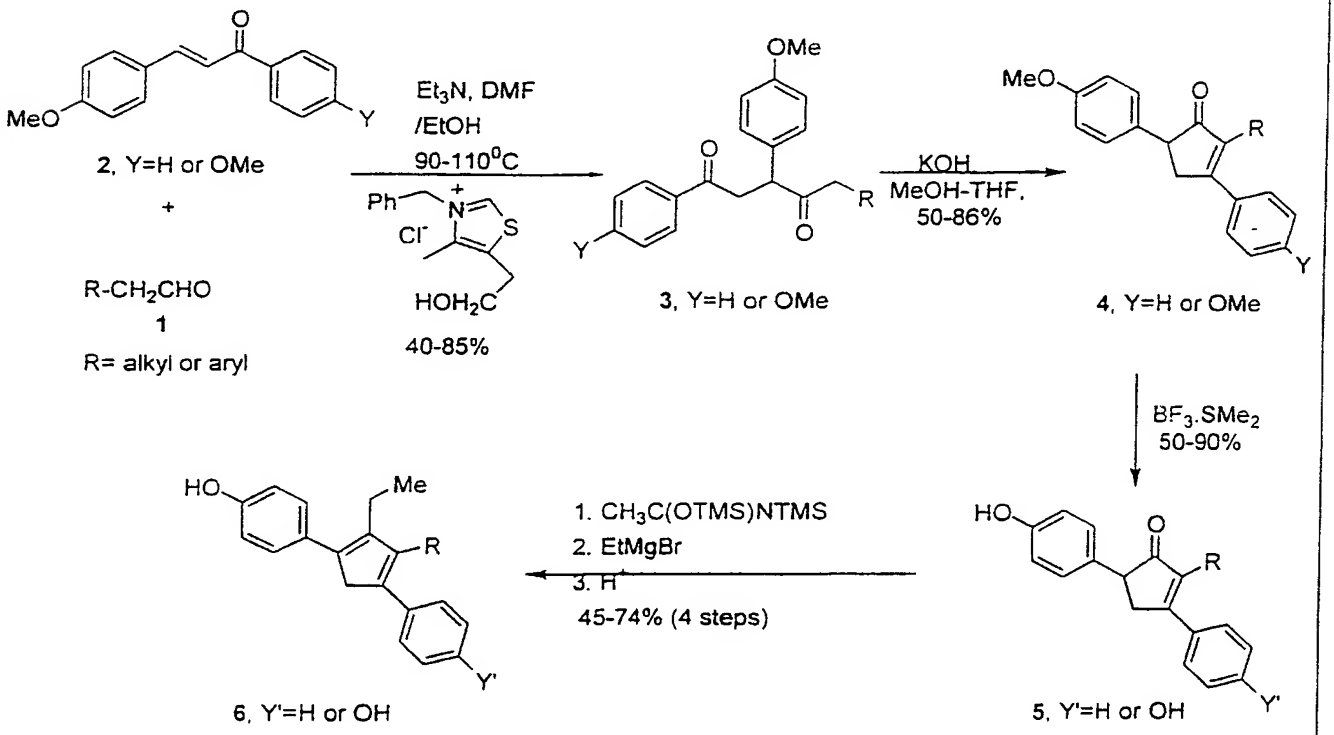
Scheme 11A



Scheme 11B

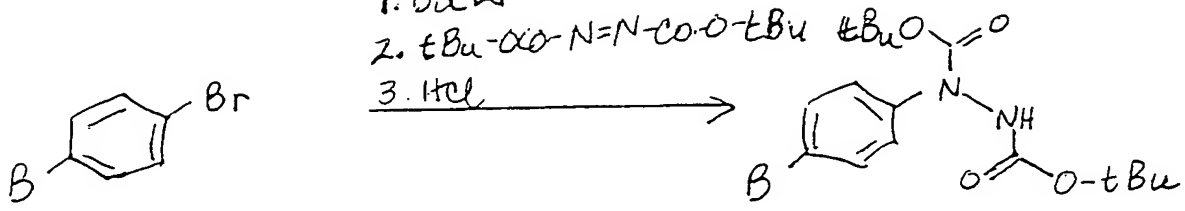


Scheme 11C: General Route for the Synthesis of Cyclopentadienes



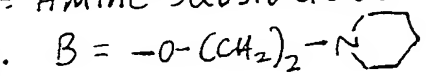
Scheme 11D: General Route for the Synthesis of Cyclopentadienones

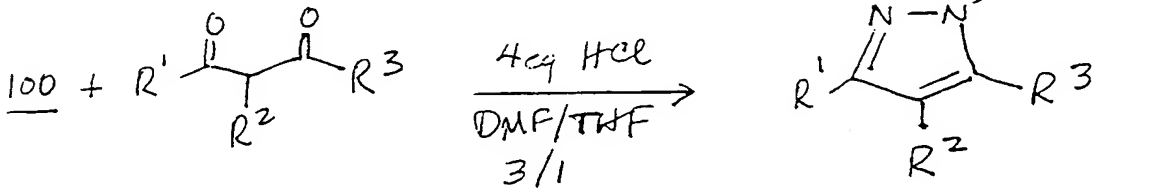
Scheme 12A



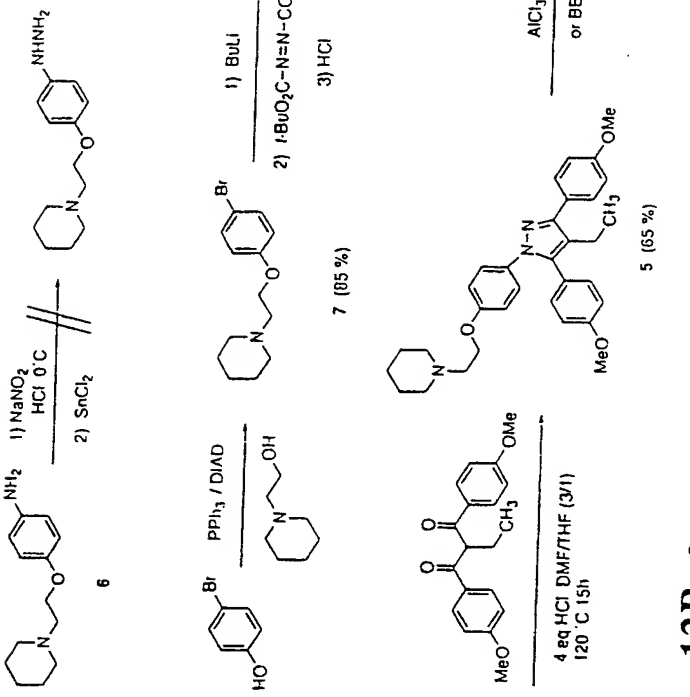
100

B = Amine substituent

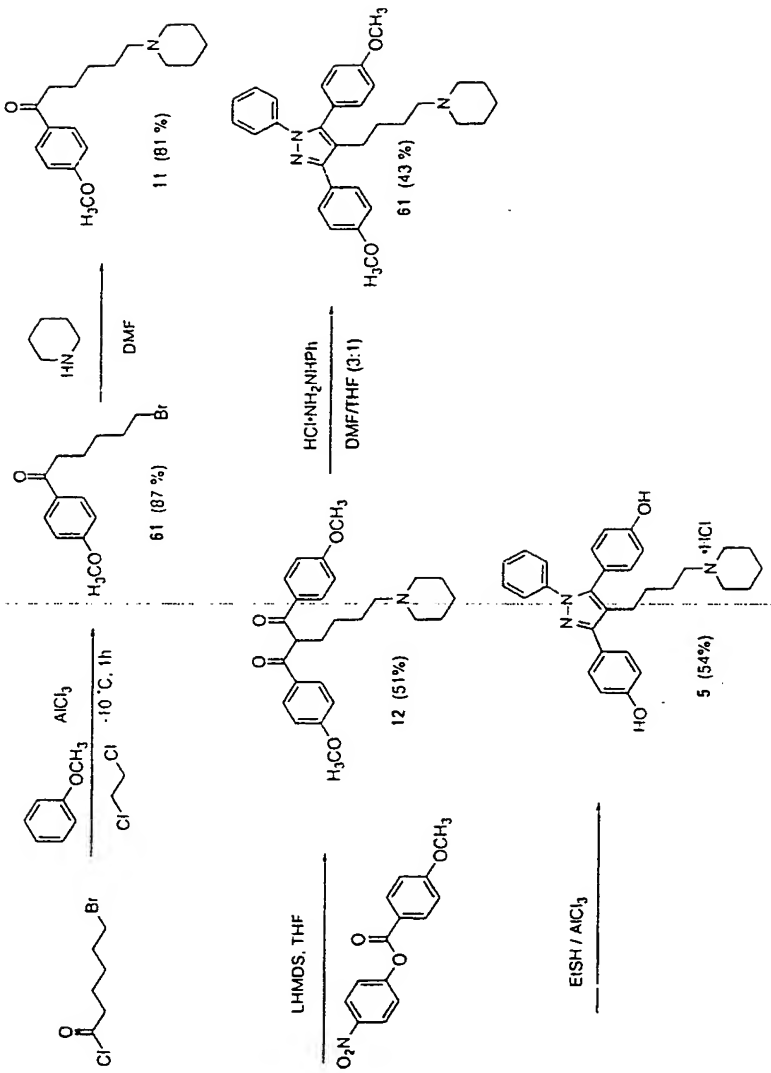
e.g. B = $-O-(CH_2)_2-N$ 



+ regioisomers



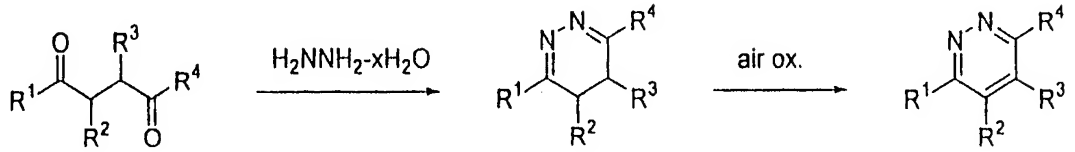
Scheme 12B. Synthesis of N(1) basic side chain containing pyrazole 2.



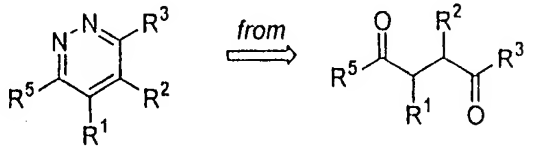
Scheme 12C. Synthesis of C(4) basic side chain containing pyrazole 3. 20

Scheme 13A

Pyridazine synthesis particularly: $R^1 = C_6H_5, C_6H_4\text{-}OR, R = CH_3, H$
$R^2 = C_6H_5, C_6H_4\text{-}OR', R' = CH_3, H$
$R^4 = C_6H_5, C_6H_4\text{-}OR'', R'' = CH_3, H$

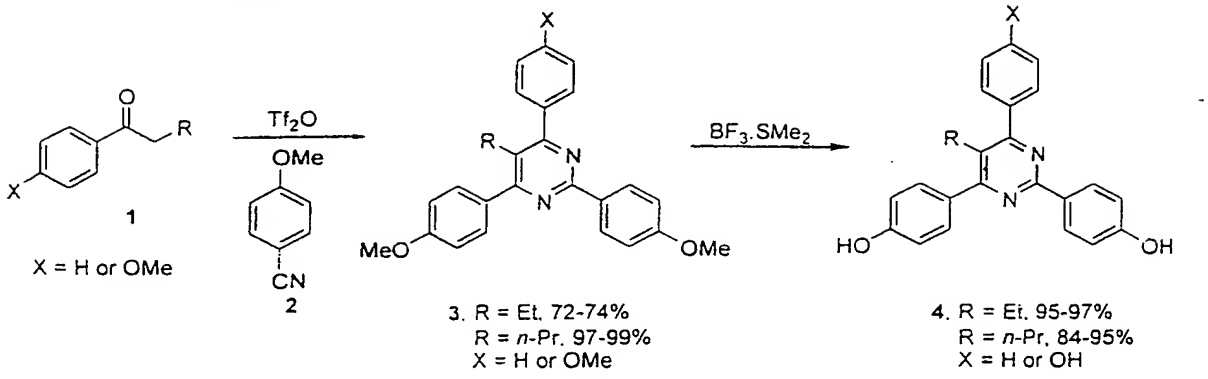


Can also be used to make:

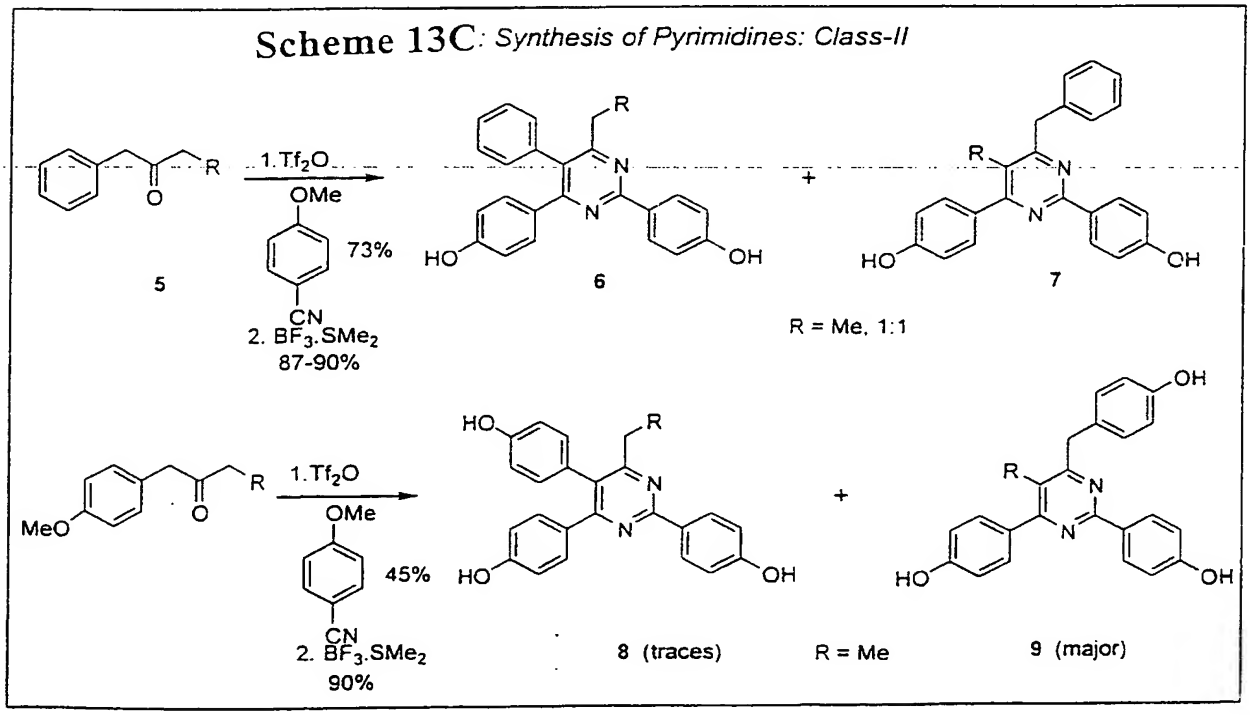


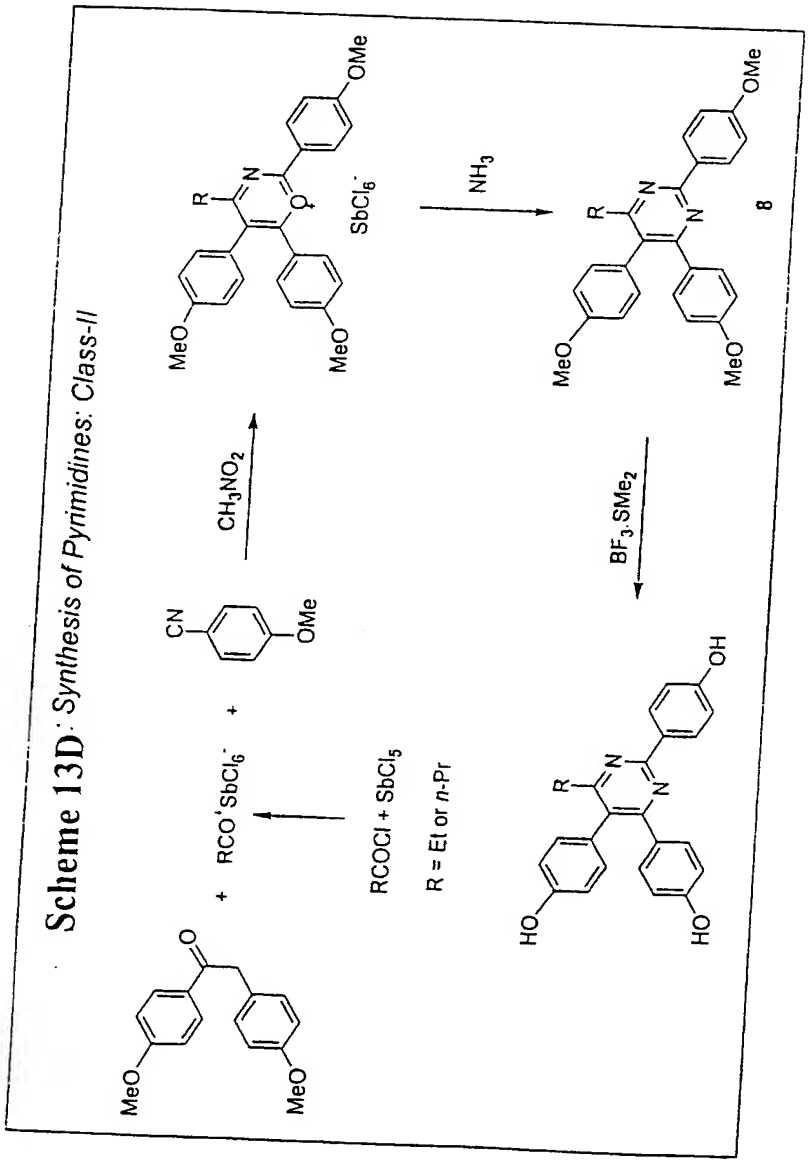
particularly: $R^1 = C_6H_5, C_6H_4\text{-}OR, R = CH_3, H$
$R^3 = C_6H_5, C_6H_4\text{-}OR', R' = CH_3, H$
$R^5 = C_6H_5, C_6H_4\text{-}OR'', R'' = CH_3, H$

Scheme 13B: *Synthesis of Pyrimidines: Class-I*

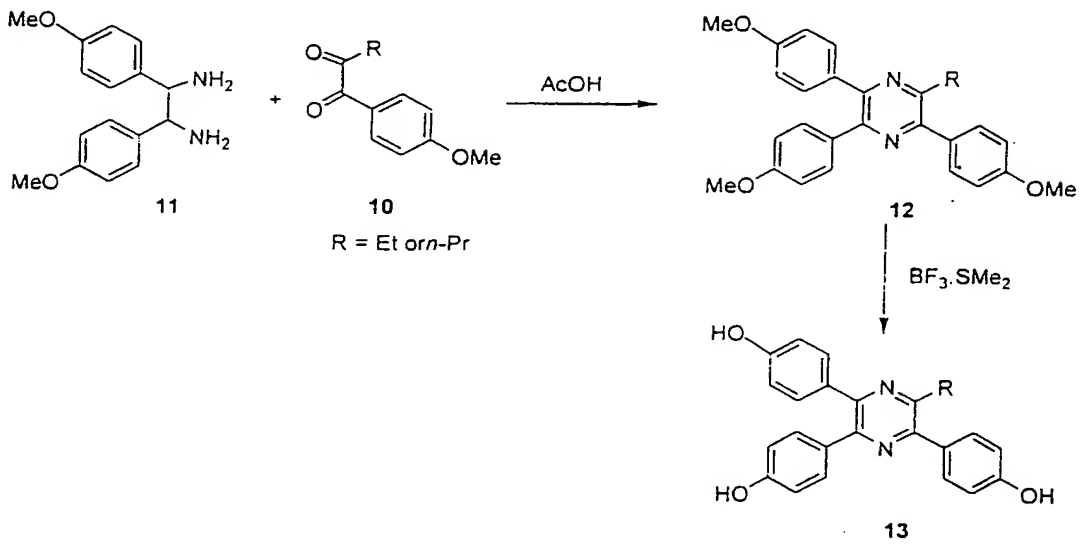


Scheme 13C: *Synthesis of Pyrimidines: Class-II*

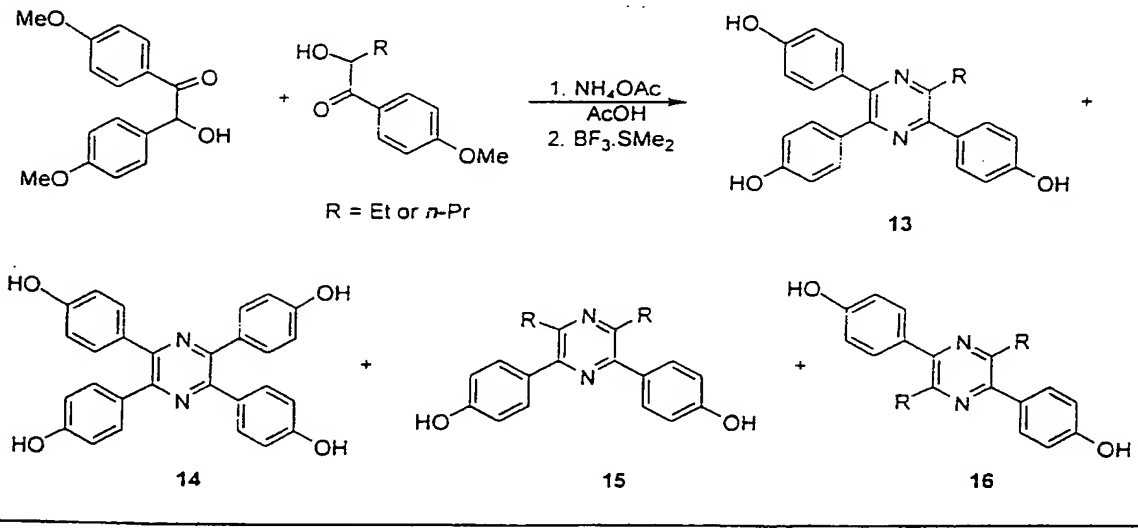




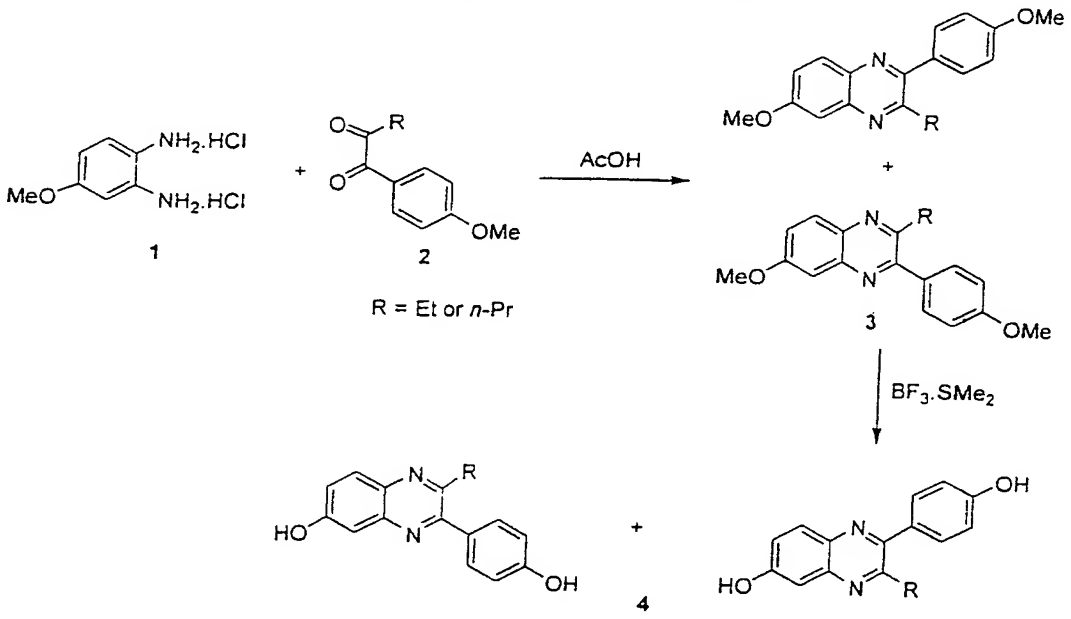
Scheme 13E: *Synthesis of Pyrazines*



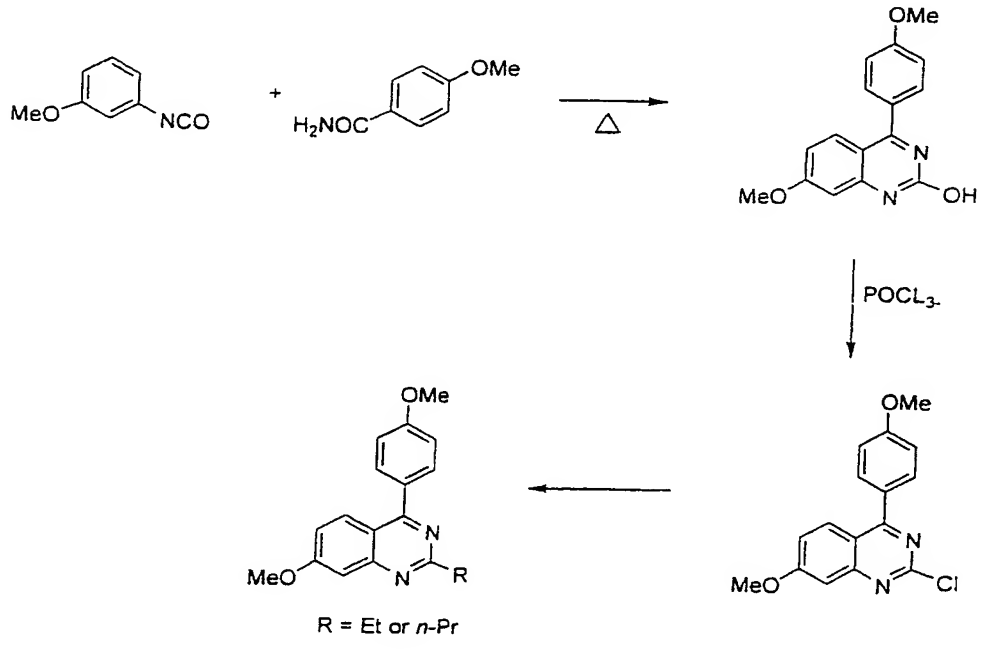
Scheme 13F: *Synthesis of Pyrazines*



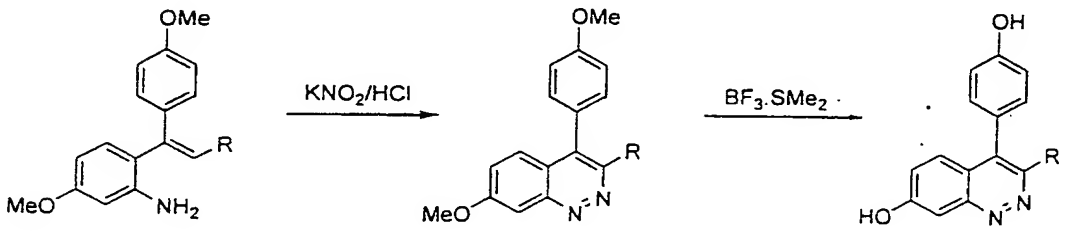
Scheme 14A: *Synthesis of Quinoxalines*



Scheme 14B: *Synthesis of Quinazolines*

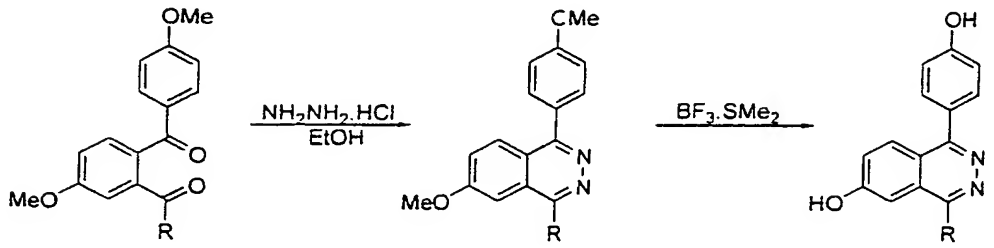


Scheme 14C: *Synthetic route to cinnolines*



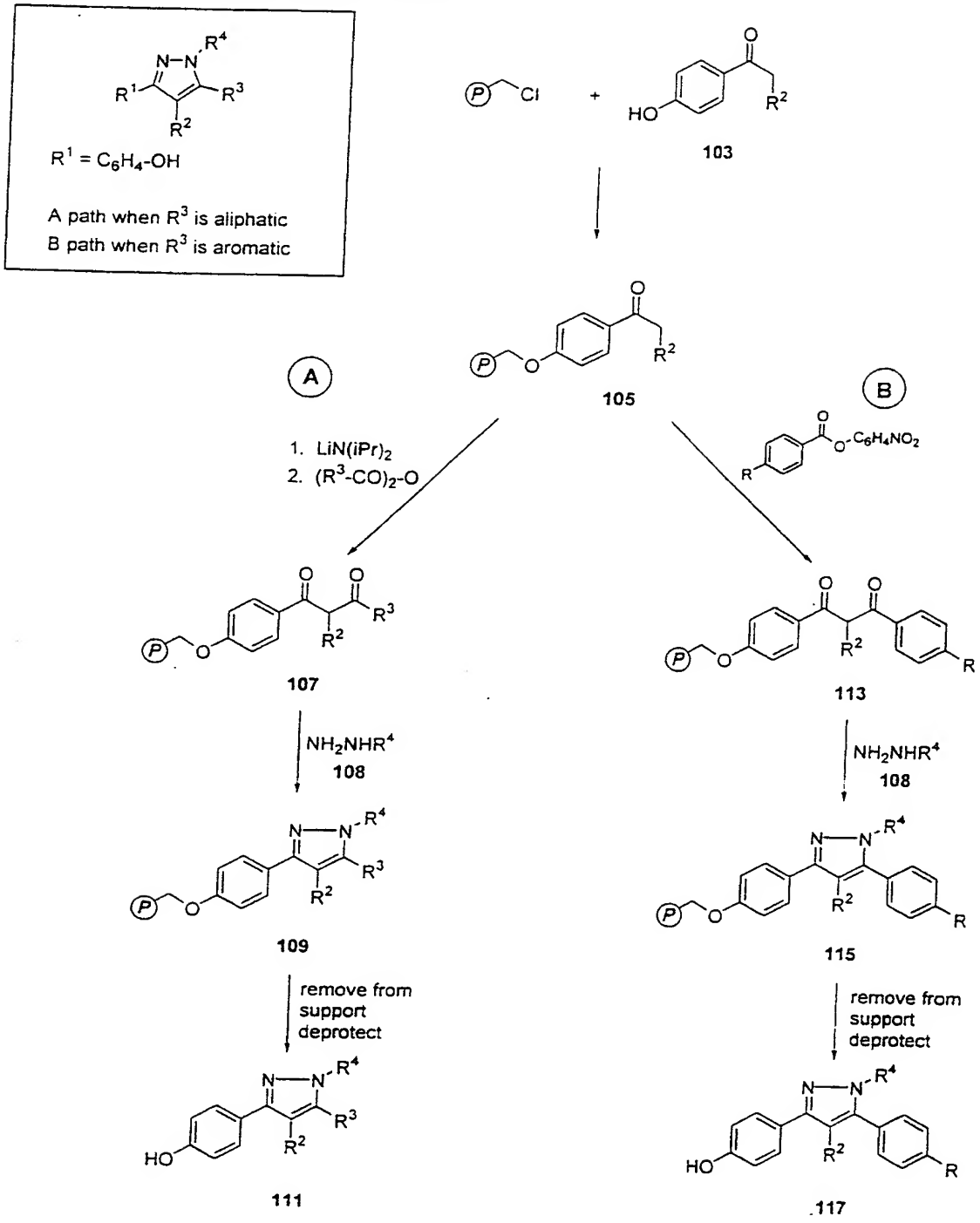
R = Et or *n*-Pr

Scheme 14D: *Synthetic route to phthalazines*

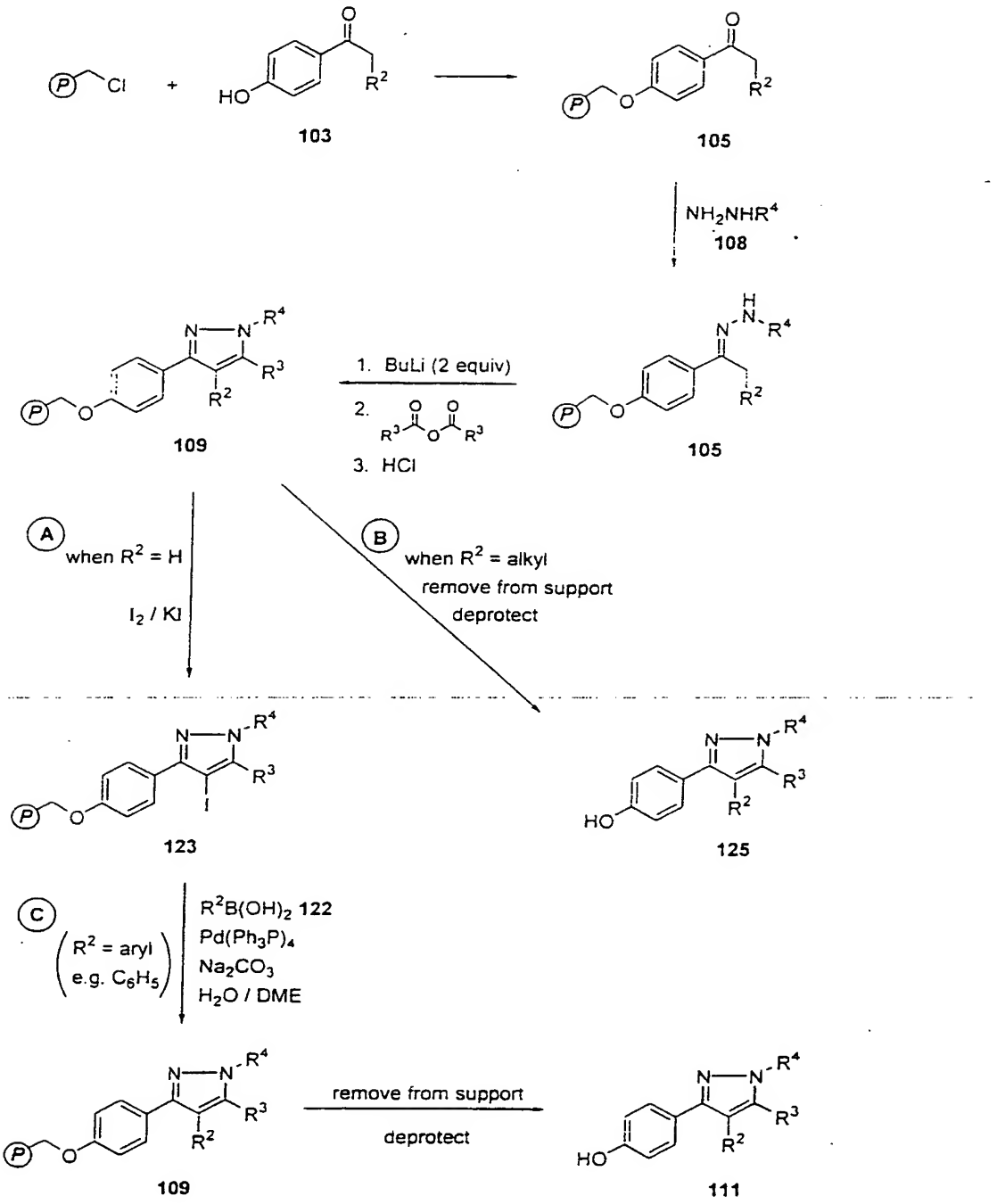


R = Et or *n*-Pr

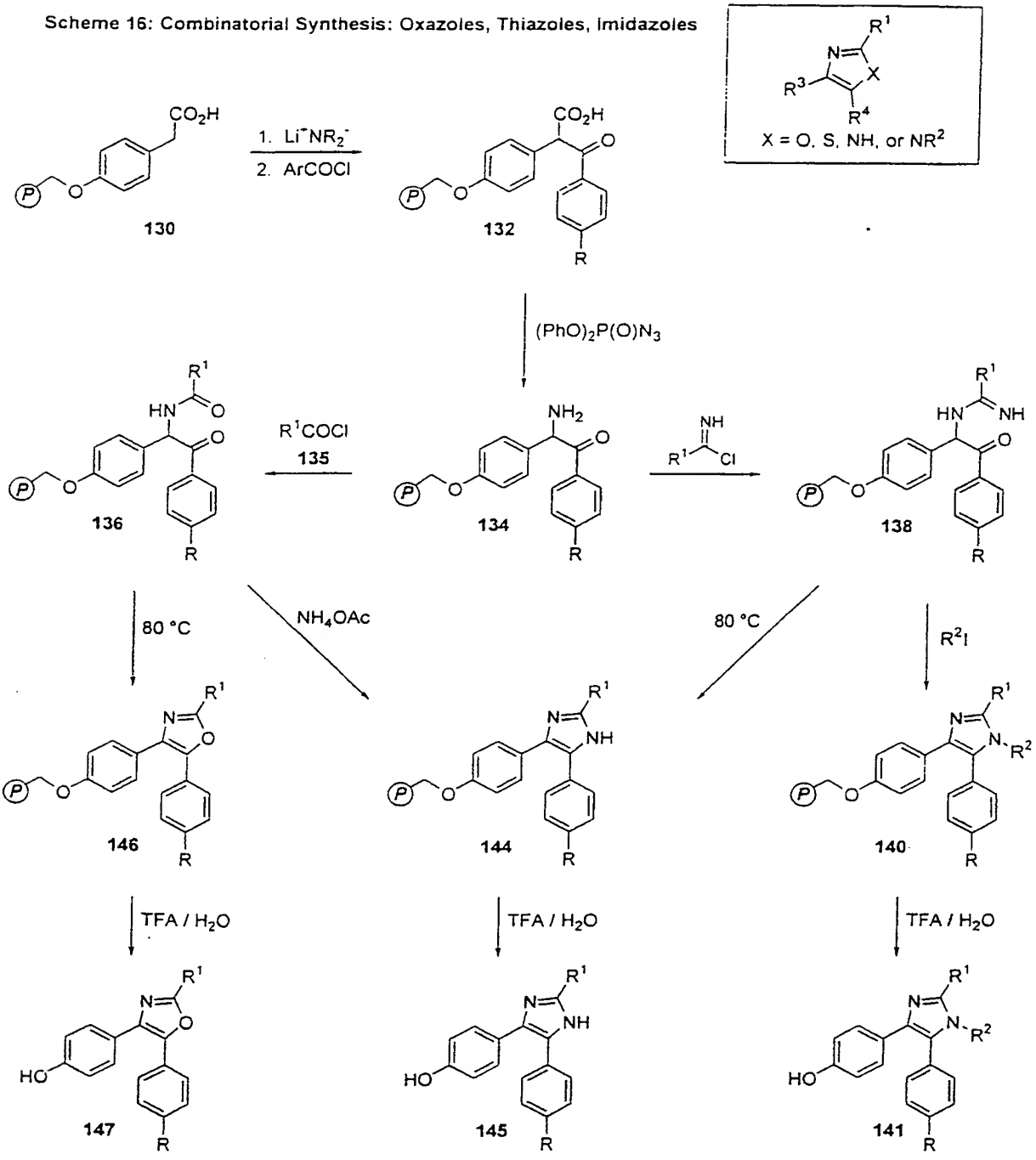
Scheme 15A: Combinatorial Synthesis: Pyrazoles



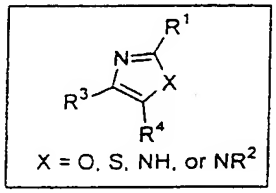
Scheme 15B: Combinatorial Synthesis: Pyrazoles(II)

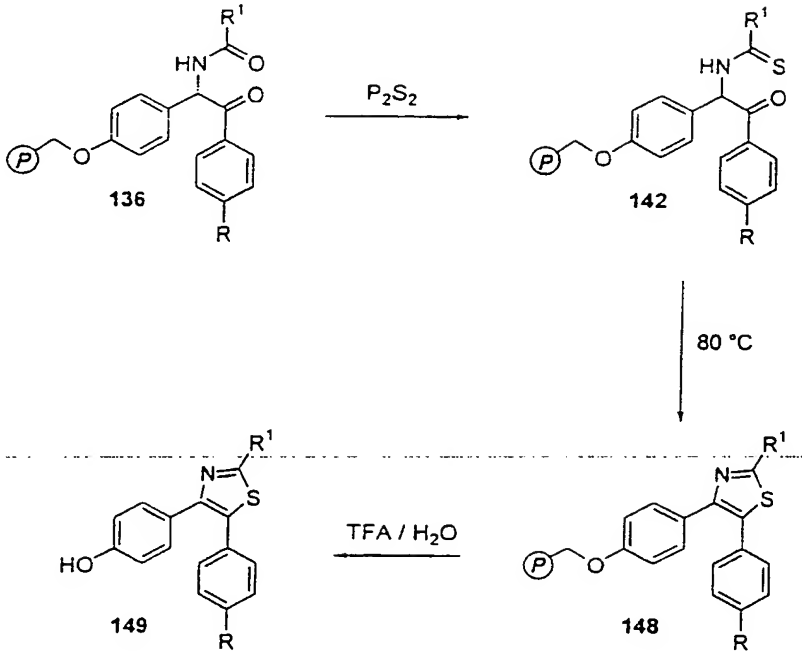


Scheme 16: Combinatorial Synthesis: Oxazoles, Thiazoles, Imidazoles

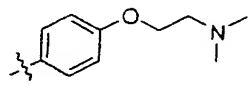


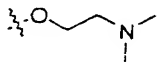
Scheme 16: Combinatorial Synthesis: Oxazoles, Thiazoles, Imidazoles (cont)

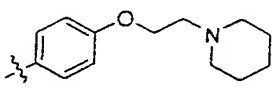


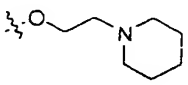


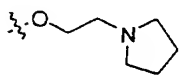
Scheme 17: Exemplary Basic and Polar Substituents

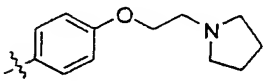


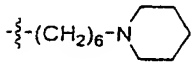


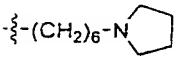


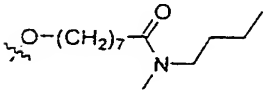


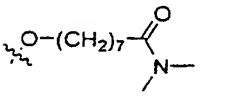


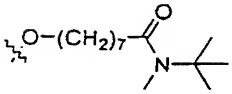


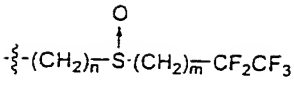


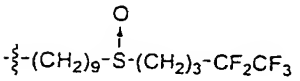


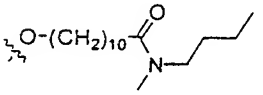


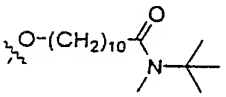


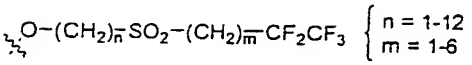


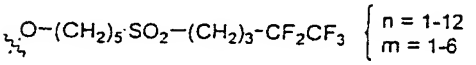




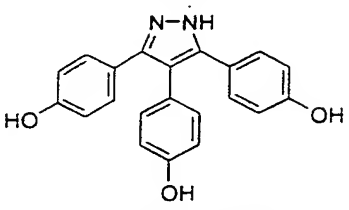




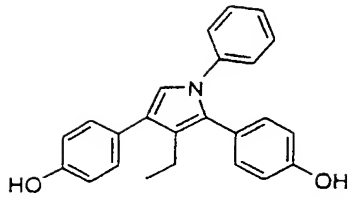


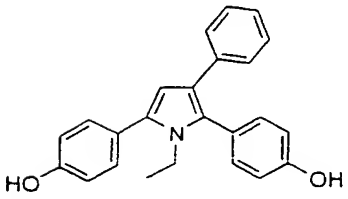


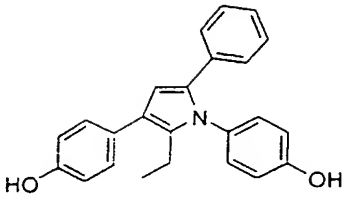
Scheme 18 : Exemplary ER Ligands

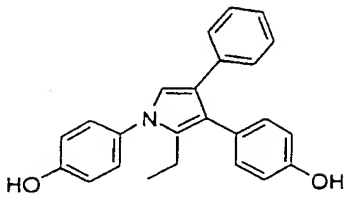


RBA = 0.016%

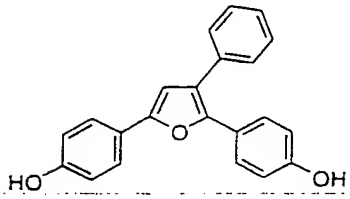




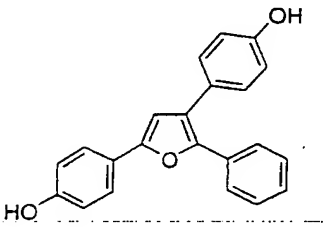




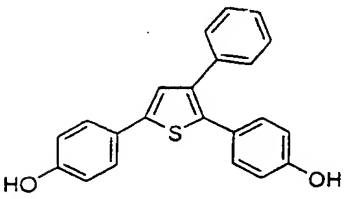
RBA = 0.50%



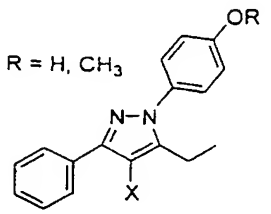
RBA = 0.05%

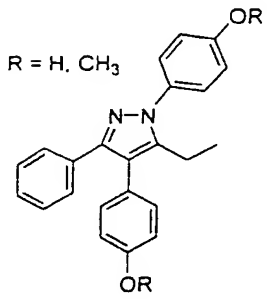


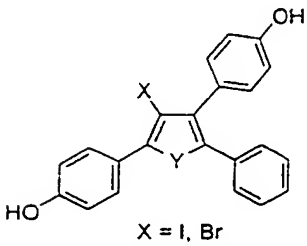
RBA = 0.135%



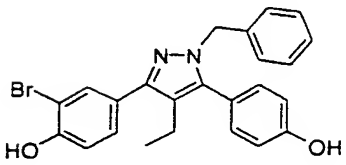
RBA = 0.03%



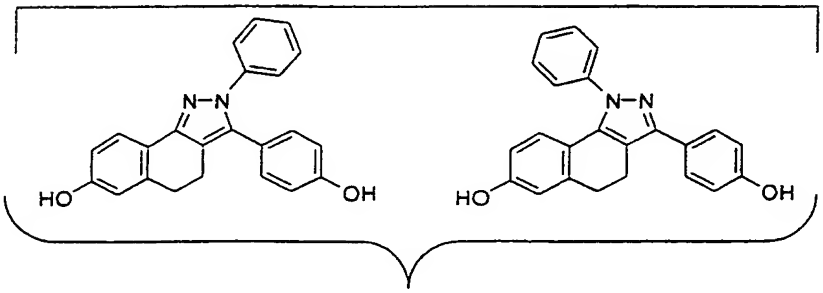




X = I, Br
R = H, CH_3
Y = O, S

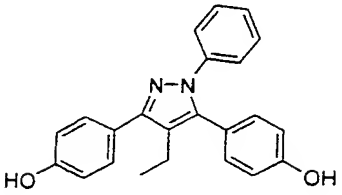


RBA = 0.12%

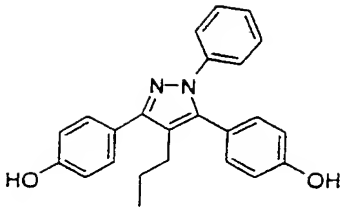


RBA = 0.68 % (1:1 mixture of isomers)

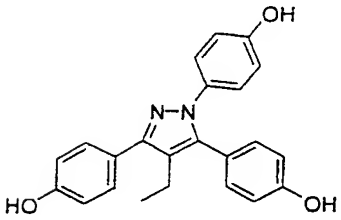
Scheme 18 (Continued)



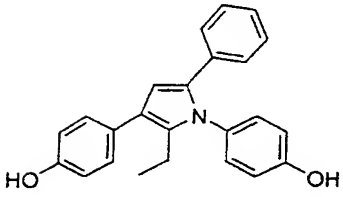
38b
RBA = 14%



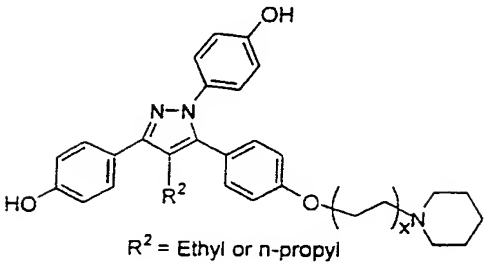
RBA = 25%



38d
RBA = 20%

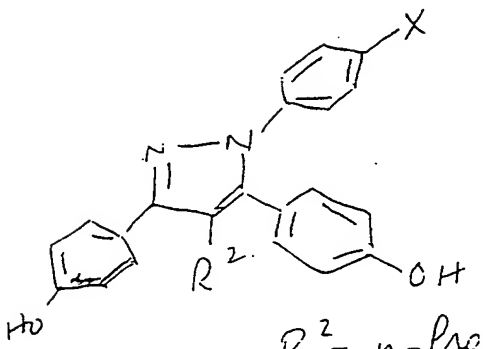


RBA = 0.50%



R^2 = Ethyl or n-propyl

Scheme 15 (continued)



334 R^2 = n-Propyl
X = H
RBA = 25
ERα = 53
ERβ = 0.54

335 R^2 = Ethyl
X = OH
RBA = 19
ERα = 31.6
ERβ = 0.30

336 R^2 = n-Propyl
X = OH
RBA = 30
ERα = 63
ERβ = 0.095

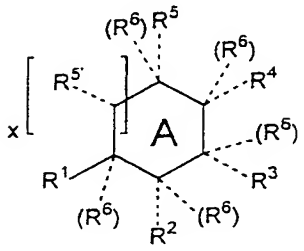
333 R^2 = Ethyl
X = H
RBA = 14
ERα = 60 ± 16
ERβ = 18 ± 4

339 R^2 = i Butyl
X = OH
RBA = 23

339d R^2 = i Butyl
X = H
RBA = 4.3

We claim:

1. An estrogen receptor ligand having the structure:



wherein x is 0 or 1 and when x = 0 the core ring A is a 5 -membered ring structure that is doubly unsaturated or when x is 1 the core ring is a 6-membered ring structure which is aromatic wherein the ring can be a carbocyclic ring or a heterocyclic ring having one or two non-carbon heteroatom and wherein:

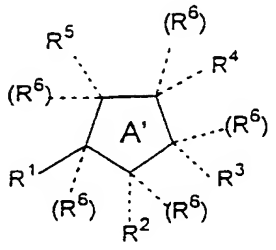
R^1 can be selected from the group consisting of phenyls and substituted phenyls wherein the non-hydrogen phenyl group substituents can include, without limitation, basic or polar groups, halogens, hydroxy groups, lower alkyl, alkenyl, alkynyl, and alkoxyl groups, lower ethers, ketones, or thioethers, and substituted lower alkyl, alkenyl or alkynyl groups, where the substituents can be halogens or hydroxy groups;

R^2, R^3 and R^4, can be the same or different, and can be selected from the group consisting of hydrogen, a basic or polar group, a phenyl or substituted phenyl group, lower alkyl, alkenyl or alkynyl where the lower alkyl, alkenyl or alkynyl groups may be substituted, with a basic or polar group, a phenyl, hydroxyls or halogens, lower ethers, ketones or thioethers, and halogens;

R^5 or $R^{5'}$, when present, can be selected from any of the groups defined for R^2, R^3 and R^4 or may be hydrogens, R^5 and $R^{5'}$ may be the same as or different than any of R^1, R^2, R^3, or R^4 and R^5 or $R^{5'}$ may be the same or different than each other; and

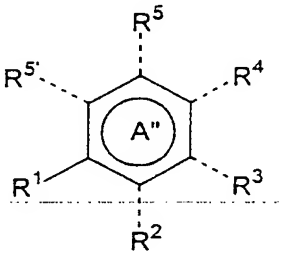
R^6, when present, can be hydrogen, a basic or polar group, a lower alkyl, alkenyl, alkynyl, or alkoxy groups, which may be substituted with lower alkyl, alkenyl or alkynyl groups, lower ether or thioethers or halogens and one or more of any $-CH_2-$ groups in R^6 can be replaced with -CO- groups.

2. The estrogen receptor ligand of claim 1 which has the structure:



wherein A' is a 5 -membered ring structure that is doubly unsaturated.

3. The estrogen receptor ligand of claim 1 which has the structure:



wherein A" is a 6-membered ring structure which is aromatic.

4. The estrogen receptor ligand of claim 1 that is a pyrazole.

5. The estrogen receptor ligand of claim 1 that is a cyclopentadiene.

6. The estrogen receptor ligand of claim 1 that is a furan.

7. The estrogen receptor ligand of claim 1 that is a pyrimidine.

8. The estrogen receptor ligand of claim 1 wherein R' is a p-OH-phenyl group.

9. The estrogen receptor ligand of claim 8 wherein R^3 is a p-OH-phenyl group.

10. The estrogen receptor ligand of claim 8 wherein R^2 is a lower alkyl group.

11. The estrogen receptor ligand of claim 8 wherein R3 is a phenyl group substituted with a basic or polar group.

12. The estrogen receptor ligand of claim 1 wherein R^2 is an ethyl or an i-propyl group.

13. A pharmaceutical composition comprising an estrogen receptor of claim 1 in an amount sufficient to exhibit an effect on a hormone-dependent disorder.

14. A method for treating a hormone-dependent disorder which comprises the step of administering to a patient suffering from that disorder the pharmaceutical composition of claim 13.

15. The method of claim 14 wherein the hormone-dependent disorder is hormone-responsive breast cancer.

16. A method for treating estrogen responsive disorders and physiological conditions which comprises the step of administering to a patient suffering from the disorder a pharmaceutical composition of claim 14.

17. A method for selective regulation of a cellular activity under the control of estrogen receptor which comprises administering a composition comprising an amount of an estrogen ligand of claim 1 sufficient to effect such cellular activity.

18. The estrogen receptor ligand of claim 1 which exhibits RBA of about1% or more.

19. The estrogen receptor ligand of claim 1 which exhibits RBA of about 10% or more.

20. The estrogen receptor ligand of claim 1 which exhibits selective affinity for one of ERα or ERβ.

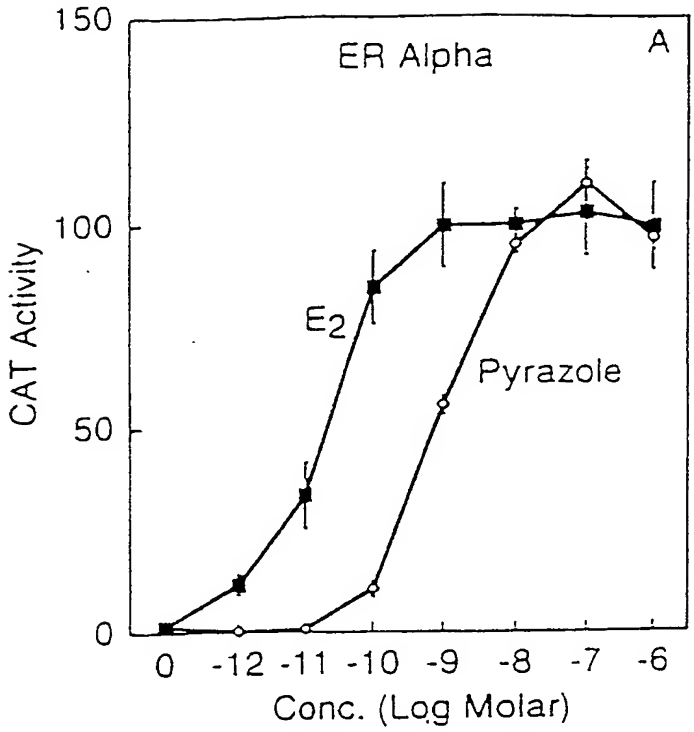


FIG. 1A

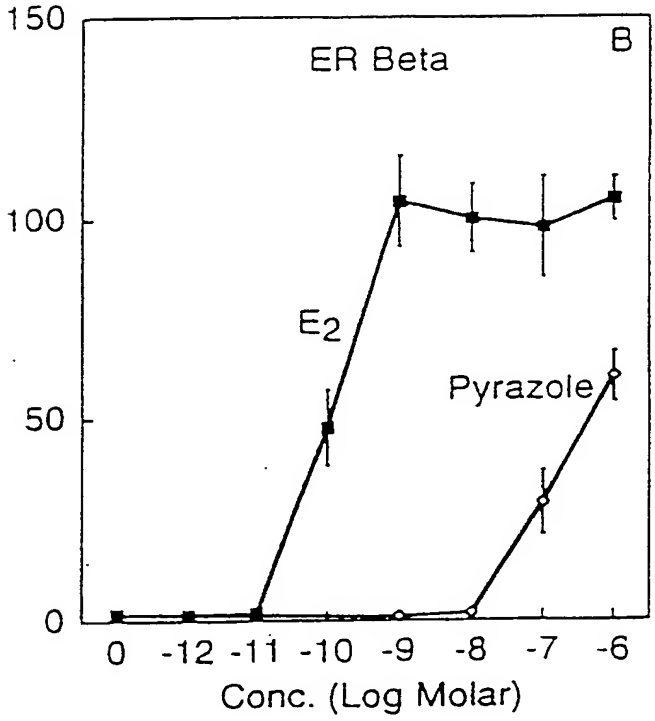


FIG. 1B

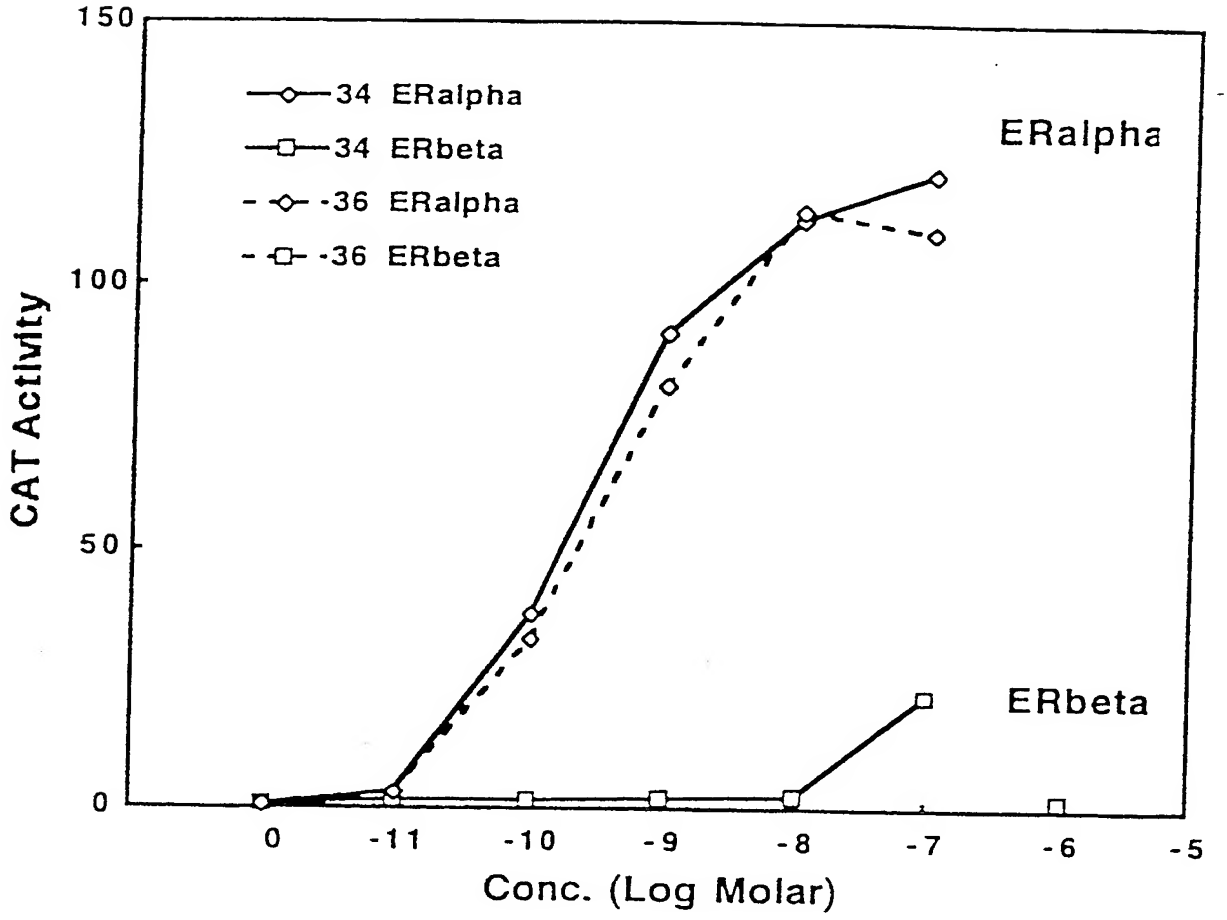


FIG. 2

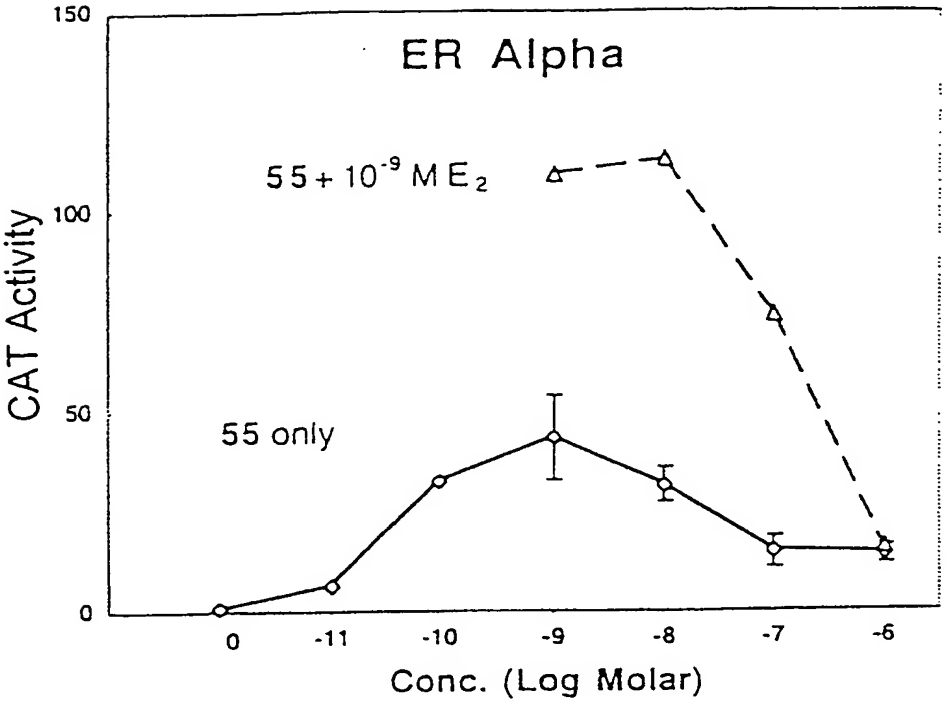


FIG. 3A

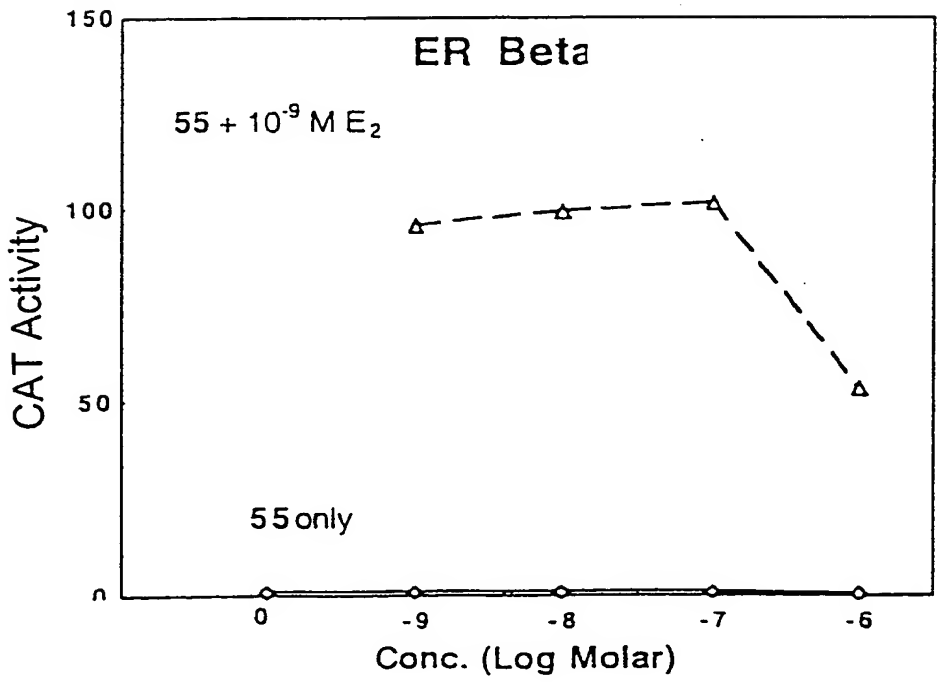


FIG. 3B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/22747

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/05, 31/341, 31/415, 31/505; C07C 39/12, 39/17; C07D 231/12, 237/08, 307/36
US CL : 514/256, 406, 461, 729, 736; 544/242; 548/377.1; 549/506; 568/731, 744

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/256, 406, 461, 729, 736; 544/242; 548/377.1; 549/506; 568/731, 744

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN/CAS, structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,629,340 A (KUWANO et al.) 13 May 1997 (13.05.97), see entire docucment.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search
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CORRECTED VERSION*

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(71) Applicant: BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS [US/US]; 506 South Wright Street, Urbana, IL 61801 (US).

(72) Inventors: KATZENELLENBOGEN, John, A.; 704 West Pennsylvania Avenue, Urbana, IL 61801 (US). KATZENELLENBOGEN, Benita, S.; 704 West Pennsylvania Avenue, Urbana, IL 61801 (US). FINK, Brian, E.; 526 Camino del Mar #36, Del Mar, CA 92014 (US). STAUFFER, Shaun, R.; 219 Nottingham, Springfield, IL 62704 (US). MORTENSEN, Deborah, S.; 502 West Main Street, Urbana, IL 61801 (US). SATTIGERI, Viswajanani, Jitendra; 2108 South Orchard Street #303, Urbana, IL 61801 (US). HUANG, Ying; Apartment 13, 1080 Baytowne Drive, Champaign, IL 61821 (US).

(74) Agents: SULLIVAN, Sally, A. et al.; Greenlee, Winner and Sullivan, P.C., Suite 201, 5370 Manhattan Circle, Boulder, CO 80303 (US).

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(54) Title: ESTROGEN RECEPTOR LIGANDS

(57) Abstract

This invention provides non–steroidal estrogen receptor ligands having a modular structure that is amenable to solid phase synthesis and the application of combinatorial synthetic methods to prepare these estrogen receptor ligands. ER ligands of this invention consist of a core scaffold that is a carbocyclic or heterocyclic–5–member ring that has two double bonds or a 6–member aromatic ring. A plurality of selected substituents are bonded to the ring substantially independently of other substituents. The modular structure of these compounds allows for synthesis of a very large numer of substituent structural variations, substituent combinations and substituent positioning on the core. The structural variants of the ER ligands of this invention exhibit a spectrum of selective affinities for ERα and ERβ and a spectrum of agonist/antagonist properties.

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ESTROGEN RECEPTOR LIGANDS

This invention was made at least in part through United States government funding through the National Institutes of Health (PHS 5R37 DK15556) and the U.S. Army (DAMD17-97-1-7076). The United States government has certain rights in this invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application takes priority under 35 U.S.C § 119(e) from U.S. provisional application serial number 60/102,881 filed October 2, 1999 which is incorporated in its entirety by reference herein.

BACKGROUND OF THE INVENTION

Estrogens are endocrine regulators of the female reproductive system that also have important effects in many non-reproductive tissues (bone, liver, cardiovascular system, CNS, etc.). Many estrogen pharmaceuticals, based on both natural and synthetic substances, have been developed as agents for regulating fertility, preventing and controlling hormone-responsive breast cancer, and menopausal hormone replacement. These substances display a spectrum of agonist to antagonist activity that can show remarkable tissue and cell selectivity [Grese, T.A. et al. (1997), "Molecular determinants of tissue selectivity in estrogen receptor modulators," Proc. Natl. Acad. Sci. USA **94**:14105-14110].

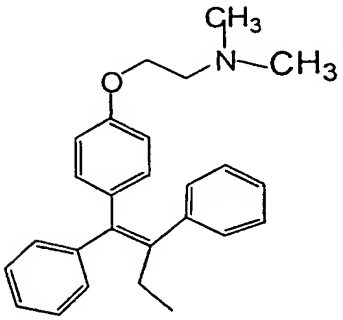
The molecular target of estrogens is the estrogen receptor (ER), of which there are now known to be two subtypes, ER-α and ER-β, that have different patterns of tissue expression and somewhat different ligand binding specificities [Mosselman, S. et al. (1996), "ERβ: Identification and characterization of a novel human estrogen receptor," FEBS Lett **392**:49-53; Kuiper, G.G. J.M. et al. (1996), "Cloning of a novel receptor expressed in rat prostate and ovary," Proc. Natl. Acad. Sci. USA **93**:5925-5930]. ER is a transcription factor that binds to specific estrogen response elements in the promoter region of estrogen-regulated genes and whose activity for transcription is modulated by the estrogen ligands [Katzenellenbogen, J.A. and Katzenellenbogen, B.S. (1996), "Nuclear hormone receptors: ligand-activated regulators of transcription and diverse cell responses," Chem. Biol. **3**:529-536]. The capacity of ER-ligand complexes to activate gene transcription is mediated by a series of co-regulator proteins [Horwitz, K.B. et al. (1996), "Nuclear receptor coactivators and corepressors," Mol. Endocrinol. **10**:1167-1177]. These co-regulators have interaction

functions that tether ER to the RNA polymerase II preinitiation complex, as well as enzymatic activities to modify chromatin structure [Glass, C.K. et al. (1997), "Nuclear receptor coactivators," Curr. Opin. Cell. Biol. 9:222-232]. Each cell type and each gene presents to an ER(subtype)-ligand complex a unique combination of these effector components – various estrogen response elements and co-regulators – that appear to underlie, in part, the cell and gene selectivity of various estrogens [Katzenellenbogen, J.A. et al. (1996), "Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones," Mol. Endocrinol. 10:119-131]. Tissue specificity and differences in agonist/antagonist activity of ER ligands may also, at least in part, be attributed to differences in ligand activity with or affinity for different sub-types of the ER receptor.

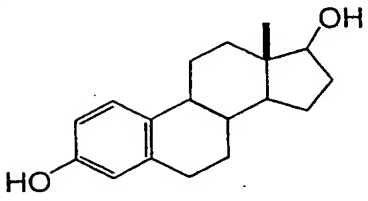
One third of all breast carcinomas are hormone-responsive and nearly all of these are estrogen-positive [Henderson, I.C., Cannellos G.P. (1980) *New Eng. J. Med.* 320:17]. For patients with estrogen-responsive tumors, hormonal therapies are preferred over cytotoxic chemotherapy and radiotherapy regimens because of their lower toxicity and the possibility that further remissions can be achieved with sequential use of multiple endocrine regimens [Royce, C. (1993) *Drugs of the Future* 18:599-600].

Among known ligands for ER, the natural estrogens are the simplest of the steroidal hormones, distinguished by having a phenolic A-ring. Synthetic estrogens, especially those of non-steroidal nature, generally retain a phenolic function (at least for those of high potency), but otherwise span a remarkable range of structural motifs that encompass simple acyclic core structures of various lengths and sizes, as well as a variety of ring-size fused and non-fused carbocyclic and heterocyclic systems [Magarian, R.A. et al. (1994), "The medicinal chemistry of nonsteroidal antiestrogens: A review," Curr. Med. Chem. 1:61-104; Solmssen, U.V. (1945), "Synthetic estrogens and the relation between their structure and their activity," Chem. Rev. 37:481-598]. Minor changes in the structure and stereochemistry of these ligands can have profound effects on both their affinity for ER, as well as their biocharacter (i.e., agonist vs antagonist balance). Major efforts have been directed toward optimizing ER ligand structure to obtain desired profiles of tissue selectivity, and even so, the ideal profile for various uses has not yet been achieved [Grese, T.A. et al. (1997), "Molecular determinants

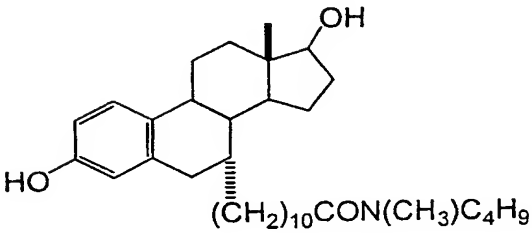
of tissue selectivity in estrogen receptor modulators," Proc. Natl. Acad. Sci. USA 94:14105-



Tamoxifen



Estradiol



ICI 164,348

14110; Grese, T.A. et al. (1998), "Synthesis and pharmacology of conformationally restricted raloxifene analogues: highly potent selective estrogen receptor modulators," J. Med. Chem. **41**:1271-1283].

Tamoxifen, the ER ligand most commonly employed in hormonal therapy for estrogen-positive breast cancer [Jordan, V.C. (1995) Breast Cancer Res. Treat. **36**:267-285], is a mixed agonist/antagonist for ER. This drug exhibits a number of side effects when used in breast cancer therapy. The level of agonist-antagonist activity of tamoxifen is variable and tissue dependent [Katzenellenbogen, B.S. (1996) *Biol.Reprod.* **54**:287-293 and Katzenellenbogen, J.A. et al. (1996) *Mol. Endocrinol.* **10** :119-131]. Tamoxifen may increase the incidence of liver and uterine cancer [Davidson, N. (1995) New Eng. J. Med. **332**.:1638-1639 and Katzenellenbogen, B.S. (1991) J. Natl. Cancer Inst.**83** :1434-1435]. In contrast, the stimulatory effects of tamoxifen in bone cells can be beneficial for the prevention of osteoporosis in postmenopausal women [Katzenellenbogen, B.S. (1996) *Biol.Reprod.* **54**:287-293]. Pure antiestrogens, such as ICI 164,384 also show promise for hormonal therapy for estrogen-positive breast cancer, but exhibit detrimental effects on other estrogen positive tissues (bone, central nervous system and the cardiovascular system). A selective endocrine profile, as yet not achieved, which effects the desired inhibitory response in targeted tumor cells, while avoiding detrimental inhibitory or stimulatory effects in other tissues, is preferred in a drug for use in hormonal therapy for estrogen-positive breast cancer.

Combinatorial chemistry is of significant current interest for the identification of drug candidates. Combinatorial synthetic methods involve the parallel synthesis of a large collection of structurally related analogs to generate a library of compounds representing systematic structural variations that is then available for functional assessment. Combinatorial libraries are most often screened for a selected biological activity or function. Assessment of the properties of the members of such libraries of structurally related compounds can provide valuable insight into the relationship between structure and the property or function assessed. Combinatorial synthetic techniques have been applied extensively to the generation of large peptide libraries [Gallop, M.A. et al. (1994) "Applications of combinatorial technologies to drug discovery. 1. Background and peptide combinatorial libraries." J. Med. Chem. **37**:1233-1251]. More recently, analogous techniques employing solid-phase organic synthesis have been applied to the development of non-peptide libraries [Bunin, B.A. and Ellman, J.A. (1992), "A general and expedient method for the solid-phase synthesis of 1,4-benzodiazepine derivatives," J. Am. Chem. Soc. **114**:10997-10998; Bunin, B.A. et al. (1994), "The combinatorial synthesis and chemical and biological evaluation of a 1,4-benzodiazepine library," Proc. Natl. Acad. Sci. USA **91**:4708-4712; Hobbs-Dewitt, S. et al. (1993), "'Diversomers': an approach to nonpeptide, nonoligomeric chemical diversity," Proc. Natl. Acad. Sci. USA **90**:6909-6913; Beebe, X. et al. (1992), "Polymer-supported synthesis of 2,5-disubstituted tetrahydrofurans," J. Am. Chem. Soc. **114**:10061-10062; Chen, C. et al. (1994), "Analogous organic synthesis of small-compound libraries: validation of combinatorial chemistry in small-molecule synthesis," J. Am. Chem. Soc. **116**:2661-2662; and Zuckerman, R.N. et al. (1994), "Discovery of nanomolar ligands for 7-transmembrane G-protein coupled receptors from a diverse (N-substituted)glycine peptoid library," J. Med. Chem. **37**:2678-2685]. Combinatorial techniques have been applied to the synthesis of pyrazoles from 1,3-diketones on solid support [Marzinzik, A.L. and Felder, E.R. (1996) Tetrahedron Lett. 37:1003] and to the synthesis of various heterocycles from α,β-unsaturated ketones [Marzinzik, A.L. and Felder, E.R.(1998) J. Org. Chem. 63:723-727]. Solid-state synthesis of tetra- and penta-substituted pyrroles has been reported [Mjalli A.M.M. et al. (1996) Tetrahedron Lett. **37**:2943-2946].

For the most part, ER ligands currently under investigation are not well suited for synthesis by combinatorial approaches, because their preparation generally involves a series

of carbon-carbon bond forming reactions that are not uniformly high yield, nor well adapted to solid phase synthetic methods. For example, combinatorial approaches using solid phase synthetic methods have been applied to the preparation of ER ligands having stilbene-like structures [Williard, R., et al. (1995) Curr. Biol. 2:45-51 and Brown, D.S. and Armstrong, R.W. (1996) J.Am. Chem. Soc. **118**:6331-6332]. However, combinatorial approaches have had limited application to the preparation of ER ligands.

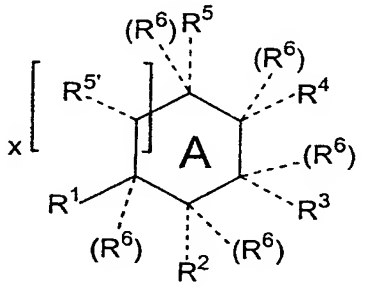
The present invention is based, at least in part, on the inventors' development of a simple modular pharmacophore for ER ligands consisting of a core structure linked to a plurality of independent substituents. The identification of this modular generic structure for ER ligands led to the development of modular stepwise synthetic methods, adaptable to solid-phase chemistry, for the generation of a combinatorial library of potential ER ligands with systematic structural variation. Structural variants are readily generated based on this pharmacophore by variation of the core structure and selection of the substituents to be linked to the core structure.

SUMMARY OF THE INVENTION

This invention provides non-steroidal estrogen receptor ligands having a modular structure that is amenable to solid phase synthesis and the application of combinatorial synthetic methods to prepare these estrogen receptor ligands. ER ligands of this invention consist of a core scaffold to which a plurality of selected substituents can be bonded substantially independently of other substituents. The modular structure of these compounds allows for synthesis of a very large number of substituent structural variations, substituent combinations and substituent positioning on the core. The structural variants produced by combinatorial methods can be assessed for differences in ER binding affinity and differences in physiological function allowing selection, for example, of ER ligands with a desired spectrum of agonist/antagonist properties. The ability to rapidly identify and select ER ligands with differences in agonist/antagonist properties allows the identification and selection of ER ligands optimized for a given clinical or pharmaceutical application.

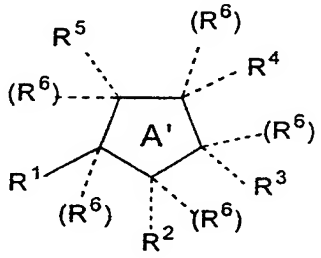
The compounds of this invention consist of a core structure that carries up to 6 substituents which together provide for binding to and interaction with ER. The core scaffold

is a 5 -membered ring structure that is doubly unsaturated (two double bonds) or 6-membered ring structure which is aromatic (triply unsaturated). The ring structure can be a carbocyclic ring or a heterocyclic ring have one or two non-carbon heteroatoms in the ring. The core ring can be described by the general formula:



where x is 0 or 1 and where the core ring A is a carbocyclic or heterocyclic ring having two double bonds, if it is a 5-membered ring (x = 0) or that is aromatic, if it is a 6-membered ring (x = 1). The positioning of double bonds and heteroatoms in the ring is not illustrated in the structure above, but various ring structures are illustrated in Tables 1 and 2. Substituents attached via dotted bonds are optional, dependent upon double bond and heteroatom placement. The substituents in parenthesis are potentially present in compounds that have 5-membered ring cores.

In one aspect, the core scaffold is a 5-membered doubly unsaturated ring structure which can be a carbocyclic ring, i.e. a cyclopentadiene, or a heterocyclic ring having one or two non-carbon elements, e.g., O, S or N, heteroatoms in the ring. ER ligands of this structure have the general formula:



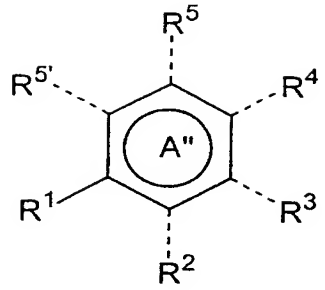
where the 5-membered ring A' can be a variety of carbocyclic and heterocyclic moieties with various positioning of two double bonds. Substituents attached with dotted lines are optional, dependent upon the position of double bonds and any heteroatoms in the ring. The possible

double bonds of ring A' and the possible heteroatoms, which can be placed in various ring positions, are not shown in the structure above. A variety of core ring A' structures illustrating placement of double bonds, heteroatoms and substituents are exemplified in Table 1.

Table 1 contains a number of exemplary core 5-membered ring structures illustrating positioning of R substituents on the 5-membered ring. Core ring structures are exemplified by cyclopentadienes, cyclopentadienones, pyrazoles, imidazoles, oxazoles, thiazoles, isoxazoles, isothiazoles, furans, pyrroles, and thiophenes. Additional structures may be obtained by varying the relative placement of substituents and double bonds. Two adjacent R substituents on the 5-membered ring can together form a cyclic structure.

Dependent upon the core structure, the position of the heteroatom(s) relative to a particular R group and/or the placement of double bonds, the core can accommodate up to 3 to 6 substituents. Substituent R^1 is required in the compounds of this invention. All 5-membered ring cores accommodate a minimum of 3 substituents (R^1 and two of R^2, R^3, R^4 and R^5). It is preferred that both R^1 and R^3 or R^1 and R^4 be present and that they are both non-hydrogen substituents. It is more preferred that R^1, R^2, and R^3 or R^1, R^2, and R^4 be present and that each is a non-hydrogen substituent. Certain cores may accommodate one (e.g., pyrroles) or two (e.g., cyclopentadienes) additional substituents, R^5 and R^6 (which can be hydrogens), on the ring atoms. Bonds to these substituents are indicated by dashed lines and each possible R^5 and R^6 is placed within parentheses to indicate that they are not present in all core structures and that the position of R^6 can be varied in certain cores. Typically there will only be one R^6 substituent in a given 5-membered ring core, which, however, may be at any ring position. In cyclopentadienones, CR^4R^6 or CR^5R^6 can represent C=O.

In a second aspect, the core scaffold is a 6-membered aromatic ring structure which can be a carbocyclic ring, i.e. a benzene, or a heterocyclic ring, e.g., a pyrazine or a pyrimidine, having one or two non-carbon elements, e.g., O, S or N, heteroatoms in the ring. ER ligands of this structure have the general formula:



where the 6-membered aromatic A" ring can be a variety of carbocyclic and heterocyclic moieties. The possible locations of heteroatoms in the ring are not shown in the above structure. One or two of the substituents attached by dotted lines can be absent dependent upon the placement of heteroatoms. A variety of core ring structures illustrating placement of heteroatoms and substituents in six-membered rings are exemplified in Table 2. Two adjacent R substituents on a 6-membered core ring can together form a cyclic structure, as illustrated by quinoxalines or quinazolines in Table 2.

Dependent upon the core structure, the position of the heteroatom(s) relative to a particular R group and/or the placement of double bonds, the aromatic core can accommodate 4 to 6 substituents. Substituent R^1 is required in the compounds of this invention. Six-membered ring cores accommodate a minimum of 4 substituents (R^1 and three of R^2, R^3, R^4, R^5 and $R^{5'}$). Substituent $R^{5'}$ is selected from the same groups as R^5 and may be the same or different from R^5. R^3 or R^1 and R^4 be present and that they are both non-hydrogen substituents. It is more preferred that R^1, R^2, and R^3 or R^1, R^2, and R^4 be present and that each is a non-hydrogen substituent. Benzenes have six substituents. Pyridines have five substituents. Pyrimidines and pyrazines have four substituents. Bonds to substituents other than R^1 are indicated by dashed lines to show that they may be absent dependent upon heteroatom placement.

Substituent R^1 can be selected from the group consisting of phenyls and substituted phenyls wherein the non-hydrogen phenyl group substituents can include, without limitation, halogens (F, Cl, and Br, being preferred), hydroxy groups, lower alkyl, alkenyl, alkynyl, and alkoxyl groups (where the term "lower" indicates 1 to about 6 carbon atoms), lower ethers, ketones, or thioethers, and substituted lower alkyl, alkenyl or alkynyl groups (where the

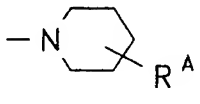
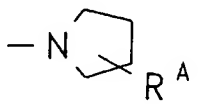
substituents can be halogens or hydroxy groups). Substituted alkyl, alkenyl and alkynyl groups can include perhalogenated groups, e.g., CF_3 or CF_2CF_3. The R^1 phenyl ring can carry multiple substituents that can be the same or different. Phenyl ring substituents can be at any of the ortho- (o-), meta- (m-) or para- (p-) positions on the ring. Preferred substituents of R^1 phenyl groups are halogens (particularly F, Cl and Br), methyl, ethyl, vinyl, methoxy, ethoxy and hydroxy groups. Preferred substituted phenyl groups are para-substituted, particularly p-halogen- and p-hydroxy- substituted phenyls. The most preferred R^1 group is p-hydroxyphenyl. In general, the R^1 phenyl group can carry any substituent that can be metabolically converted into a p-OH group, e.g., OCH_3, $O\text{-}COCH_3$, etc.

Substituents R^2, R^3 and R^4, can be the same or different, and can be selected from the group consisting of hydrogen, a phenyl or substituted phenyl group (where phenyl substitution is as described for R^1), lower alkyl, alkenyl or alkynyl (where the lower alkyl, alkenyl or alkynyl groups may be substituted, with a phenyl, hydroxyls or halogens), lower ethers, ketones or thioethers, and halogens (F, Cl, Br, or I). Where a halogen is directly attached to the core ring, Br and I are preferred halogens. Substituted alkyl, alkenyl and alkynyl groups can include perhalogenated groups, e.g., perfluorinated groups, such as CF_3 or CF_2CF_3. Substituted alkyl, alkenyl and alkynyl groups include those substituted with a phenyl ring or a substituted phenyl ring, e.g., benzyl, p-hydroxybenzyl, m-fluorobenzyl, etc. Preferred R^2, R^3, R^4 are lower alkyl or alkenyl groups, and phenyl and o-, m-, or p-substituted phenyl groups. More preferred R^2 are ethyl and propyl (straight-chain or branched) groups. More preferred R^4 are phenyls, o-, — or p-hydroxyphenyl, o-, m-, or p-alkoxyphenyl, o-, m-, or p-halophenyl, and branched alkyl groups, e.g., a t-butyl group. More preferred R^3 are phenyls and substituted phenyls, including o-, m-, or p-hydroxyphenyl, o-, m-, or p-alkoxyphenyl, o-, m-, or p-halophenyl.

Substituents R^5 or $R^{5'}$ when present, e.g., in pyrroles and cyclopentadienes and other 5-member ring systems (Table 1) or aromatic systems (Table 2), can be selected from any of the groups defined for R^2, R^3 and R^4 and may be hydrogens. R^5 and $R^{5'}$ may be the same as or different than any of R^1, R^2, R^3, or R^4. Substituents R^5 or $R^{5'}$ may be the same or different than each other.

R^6 when present, e.g. in cyclopentadienes, can be hydrogen, lower alkyl, alkenyl, alkynyl, or alkoxy groups, substituted lower alkyl, alkenyl or alkynyl groups, lower ether or thioethers or a halogen (particularly F, Cl and Br). Substitution of alkyl, alkenyl and alkynyl R^6 groups can include halogen and hydroxy group substitution. One or more of any $-CH_2-$ groups in R^6 can be replaced with -CO- groups. R^6 is preferably a lower alkyl or hydrogen.

In specific embodiments, the R^1-R^4 (and R^5, $R^{5'}$ or R^6 when present) can also carry or be basic or polar groups, e.g., an alkyl, phenyl or other substituent listed above that carries a basic or polar substituent or basic or polar groups directly linked to the core ring structure. Basic and/or polar groups on ER ligands can provide the ligand with antagonist or mixed agonist/antagonist properties. Basic groups include without limitation: amines, and amine-substituted alkyl, alkenyl or alkoxy groups. Amines can be alkyl, alicyclic or aromatic amines. Basic groups specifically include: $-(X)_x(CH_2)_n$-NRR' where X is O or S, x is 0 or 1, n is an integer from 1 to about 10 and preferably 2 to 6, and R and R', can be the same or different and can be alkyl, aryl, or alicyclic. R and R' in these specified basic groups can together form an heterocyclic or a substituted heterocyclic ring, e.g.,


or

,

where R^A (which may represent multiple substituents) can be lower alkyl, alkenyl or alkynyl groups or substituted lower alkyl, alkenyl or alkynyl groups. Additionally, any alicyclic rings can contain one or more carbonyl groups -CO-.

In general any polar groups can be employed as substituents on any R groups or for direct linkage to the ring. Preferred polar groups include halogens, perhalogenated alkyl, alkenyl or alkynyl groups, hydroxy groups, hydroxy-substituted alkyl, ethers or thioethers, diols, amides, sulfoxides, and sulfones, e.g.,:

Diols: $-(X)_x(CH_2)_n$-CH(OH)-CH(OH)-R^B where X is O or S, x is 0 or 1, n is an integer from 1 to about 6 and preferably 1 to about 4, R^B is H or $-(CH_2)_m-CH_3$, where m is an integer from 0 to about 6. Diols can also be alicyclic;

Amides:

$-(X)_x(CH_2)_nCO-NRR'$ where X is O or S, x is 0 or 1, n is an integer from 1 to about 12 and preferably 6 to about 10, and R and R', can be the same or different and can be alkyl, aryl, alicyclic, substituted alkyl, substituted aryl or substituted alicyclic, or together form an heterocyclic or a substituted heterocyclic ring; and

Sulfoxides:

$-(X)_x(CH_2)_nSOR'$ where X is O or S, x is 0 or 1, n is an integer from 1 to about 12, including those with n = 1 to about 5 and those with n= 6 to about 10, and R' can be alkyl, aryl, alicyclic, substituted alkyl, substituted aryl or a substituted alicyclic ring where substituents include halogens, particularly F, Cl and Br and perhalogenated alkyl groups, such as CF_3 and CF_2CF_3;

Sulfones:

$-(X)_x(CH_2)_nSO_2R'$ where X is O or S, x is 0 or 1, n is an integer from 1 to about 12, including those with n = 1 to about 5 and those with n= 6 to about 10, and R' can be alkyl, aryl, alicyclic, substituted alkyl, substituted aryl or a substituted alicyclic ring where substituents include halogens, particularly F, Cl and Br and perhalogenated alkyl group, such as CF_3 and CF_2CF_3.

See Scheme 17 for exemplary basic and polar substituents.

Each of the hydroxy-substituted alkyls and the above-listed amides, diols, sulfoxides and sulfones can be directly attached to the 5-membered core ring as an R^2-R^6 or $R^{5'}$ substituent or can be a substituent on any of R^1-R^6 or $R^{5'}$.

In five-membered ring compounds any two substituents on a given ring carbon can be linked to form a spiro-ring. For example, substituents R^5 and R^6 or R^4 and R^6 on the same ring atom can together form a carbon chain $-(CH_2)_n-$ where n is 3 to about 6 to form a spiro ring system with the parent cycle A. Carbons in the R^5/R^6 chain or R^4/R^6 chain may also be substituted, e.g., with halogens or lower alkyl, alkenyl or alkynyl groups, and one or two of the CH_2 groups of the chain may be replaced with an -CO-, -O- , -S- or an -NH-.

Substituents on adjacent ring atoms, e.g., the pairs R^1 and R^2, R^2 and R^3, R^3 and R^4, R^1 and R^5, $R^{5'}$ or R^6, can be linked to form a saturated or unsaturated carbocyclic or heterocyclic ring structure fused to the parent cycle A, e.g., an alkyl substituent of R^2 can be linked to a phenyl substituent at R^1 or R^3.

Substituents are generally selected independently of core ring size, as discussed above to achieve desired ER ligand characteristics, but are preferably also selected to provide stable compounds and facilitate ease of preparation.

In specific embodiments, 5- and 6-member ring ER ligands of this invention can contian two substituted or non-substituted phenyl rings in addition to R^1. In other specific embodiments, R^1 is substituted at a ring atom directly adjacent to a ring atom substituted with a lower alkyl group, particularly an ethyl, n-propyl, i-propyl, i-butyl or n-butyl group. In other specific embodiments, R^1 is a p-substituted phenyl group, where the substituent is OH or OR where R is a lower alkyl group, R^2 is a lower alkyl group (up to C6) and the ligand contains in addition one or two substituted or non-substituted phenyl groups. Preferred substituents on the additional phenyl rings are p-OH, m-OH, p-halogen, m-halogen, p-OR or m-OR where R is a lower alkyl group.

In more preferred embodiments, the ER ligands of this invention have core structures as listed in Tables 1 and 2 where the substituents R^1-R^6 and $R^{5'}$ are as defined above. Structural variants in addition to those listed in Table 1 and 2 may be obtained by interchanging the positions of R^2, R^3, R^4, and R^5. ER ligands are those compounds which exhibit measurable binding affinity for the estrogen receptor in assays as described herein.

Exemplary compounds of this invention are provided in Scheme 18.

The non-steroidal ER ligands of this invention are useful in pharmaceutical compositions for the treatment of hormone-responsive disorders. The non-steroidal ER ligands of this invention are particularly useful in pharmaceutical applications for treatment of estrogen-responsive disorders and conditions, as active ingredients of pharmaceutical compositions in combination with a pharmaceutically acceptable carrier or exipient. The ER

ligands may be combined with each other to achieve a desired pharmaceutical response or administered in combination with known estrogens or antiestrogens. The ER ligand is present in the pharmaceutical compositions in an amount, or in combination with other ligands in a combined amount, sufficient to induce or inhibit estrogen response. In those cases in which the ER ligand selectively interacts with an ER subtype or variant, the amount of ligand (or combined amount of ligands) present in the pharmaceutical composition is in the range that induces or inhibits the desired selective response. The invention also relates to methods of treating estrogen responsive disorders and physiological conditions employing pharmaceutical compositions comprising ER ligands of this invention alone or in combination. This invention provides pharmaceutical compositions which comprise one or a mixture of ER ligands having structures disclosed herein in combination with a pharmaceutically acceptable carrier appropriate for the pharmaceutical application and compatible with the ER ligand. ER ligands are present in these pharmaceutical compositions in an amount or in a combined amount sufficient to elicit a measurable positive effect on a symptom or condition associated with an estrogen-dependent disorder on administration to an individual suffering from the symptom or disorder.

Pharmaceutical compositions of this invention can also include other steroid or non-steroid ER ligands which may supplement or enhance the activity of the composition for a particular medical application. Pharmaceutical compositions of this invention include those which are useful in the prevention and treatment of hormone-dependent cancers, including breast cancer, those useful for hormone-replacement therapy, those useful in the treatment of infertility, those useful for treatment of osteoporosis and those useful for providing cardiovascular, CNS (suppress hot flashes, provide cognitive improvements, etc.) or related benefits. Pharmaceutical compositions of this invention can be provided in a variety of dosage forms including without limitation pills for oral administration, solutions or emulsions for oral administration or for injection.

This invention also provides methods for the treatment of hormone-dependent disorders, including the treatment of hormone-responsive breast cancer, which comprise the step of administering to a patient having the disorder or symptoms thereof a pharmaceutical composition comprising one or a mixture of the ER ligands of this invention where the ER

ligand or mixture of ligands is present in the composition at a level or a combined level sufficient to effect a positive biological response.

ER ligands of this invention can exhibit agonist, antagonist or mixed agonist/antagonist function in vitro and in vivo. These functions can be assessed for a given ER ligand or ligand mixture employing in vitro methods known in the art or as described in the Examples herein. This invention also provides methods for generation of and testing of combinatorial libraries of potential ER ligands for ER binding affinity as well as for the assessment of agonist/antagonist character of a given ER ligand.

The ER ligands of this invention are useful in vitro and/or in vivo for selective activation or repression of expression, dependent upon the agonist or antagonist nature of the ligand, of a gene regulated by ER. Gene activation or repression can be selective with respect to subtype of ER (e.g., ERα or ERβ), or variant of ER (e.g., splice variant forms, truncated or processed forms, covalently modified forms, etc.).

The ER ligands of this invention are also useful in vitro and/or in vivo for selective regulation of cellular activities under the control of ER. Cellular activities may be regulated in a variety of ways by ER, subtypes of ER or variants of ER, e.g., up or down regulation of a given cellular process. Regulation is selective with respect to subtype of ER (e.g., ERα or ERβ), or variant of ER (e.g., splice variant forms, truncated or processed forms, covalently modified forms, etc.). Cellular activities that may be regulated include both genomic (related to gene expression) or non-genomic activities (not directly related to gene expression, e.g., such as regulation of calcium flux, particularly in bone cells, hormone release, particularly prolactin release from pituitary cells, etc.).

The subtype-selective ER ligands of this invention can also be of general use in the investigation of ER and its functions. These ligands can be employed to better understand structure and conformation of ER (both subtypes) and to elucidate how ER subtypes interact with other molecules and to relate structure, conformation and interaction with other molecules to ER function.

BRIEF DESCRIPTION OF THE DRAWINGS

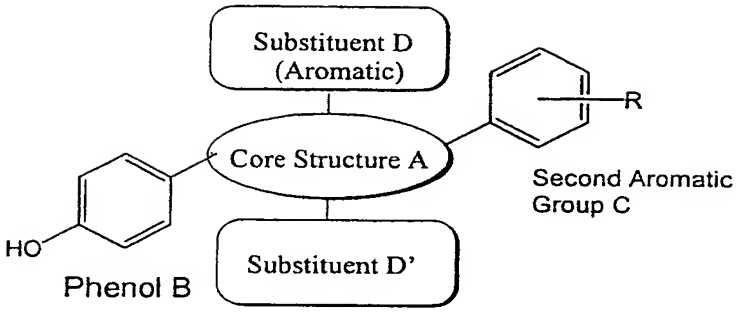
Figures 1A and 1B illustrate transcriptional activation by ERα and ERβ, respectively, in response to the pyrazole compound **38b**. Human endometrial cancer (HEC-1) cells were transfected with expression vectors for ERα (Fig. 1A) or ERβ (Fig. 1B) and an (ERE)3-pS2-CAT reporter gene and were treated with the indicated concentrations of estradiol (E2) or the pyrazole for 24 h. Cat activity was normalized for β-galactosidase activity from an internal control plasmid. Values are the mean ± SD for three or more separate experiments, and are expressed as a percent of the ERα and ERβ response with 10 nM E2. See: J. Sun et al. (1999) Endocrinology **140** (2):800-804.

Figure 2 illustrates transcriptional activation by ERα and ERβ in response to two pyrazoles XXX (solid lines) and XXX1(dashed lines). HEC-1 cells were transfected with expression vectors for ERα (diamonds) and ERβ (squares) and an $(ERE)_3$-pS2-CAT reporter gene and were treated with indicated concentrations of ligand for 24 h. CAT activity was normalized for β-galactosidase activity from an internal control plasmid. Values are the mean ± SD for three or more separate experiments, and are expressed as a percent of the ERα and ERβ response with 1 nM E2.

Figures 3A and 3B are transcriptional activation profiles for ERα (Fig. 3A) and ERβ (Fig. 3B) in response to pyrazole XXXX. HEC-1 cells were transfected with expression vectors for ERα or ERβ and an $(ERE)_3$-pS2-CAT reporter gene and were treated with indicated concentrations of ligand for 24 h in the presence or absence of estradiol (1 nM). CAT activity was normalized for β-galactosidase activity from an internal control plasmid. Values are the mean ± SD for three or more separate experiments.

DETAILED DESCRIPTION OF THE INVENTION

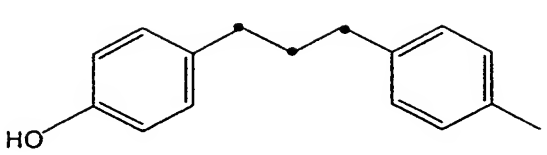
The pharmacophore model for ER ligands of this invention consists of a core structure onto which independent peripheral structural elements are attached. A preferred pharmacophore is illustrated in which a phenolic unit (B) that is always preserved, a second aromatic group (C) that is usually present, and another substituent (D) or two (D'), one of which may be aromatic is attached to the core (A):



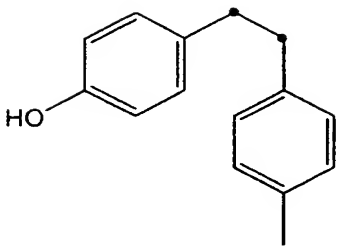
High ER binding affinity will be associated with certain geometric arrangements of the peripheral substituents (B-D'), so that they will be "in register" with their corresponding subsites in the ligand binding pocket in ER. Peripheral group orientation can be accomplished by core elements that encompass some structural variety. The core serves as a molecular scaffold whose function is to correctly orient the peripheral substituents with appropriate topology for high affinity ER binding. Further, the chemical nature of the core may effect the binding affinity and/or influence the interaction of substituents with ER.

In part because they allow some flexibility in orientation of substituent groups and in part because their synthesis is amenable to solid-phase methods, five-member and six-member carbocyclic and heterocyclic rings were selected for preparation of ER ligands based on the illustrated pharmacophore.

Two substructural motifs noted in known ER ligands are the homobibenzyl motif A, exemplified in the known non-steroidal ligands benzestrol and raloxifene and the syn-bibenzyl motif B. The A motif can be represented in various 3,5-diaryl-1,2-azoles (pyrazoles and isoxazoles) and various 2,4-diaryl-1,3-azoles (imidazoles, thiazoles, and oxazoles). The B motif can be represented in various 4,5-diaryl-1,3-azoles, as well as various 3,4-diaryl-1,2-azoles and various 4,5-diaryl-1,2-azoles. The structure of ER ligands of this invention expand from these basic motifs.



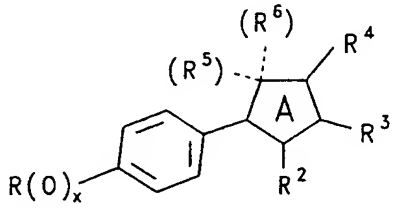
Motif A. Homobibenzyl



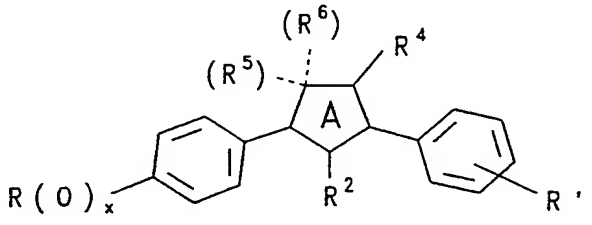
Motif B. Syn-Bibenzyl

Tables 1 and 2 illustrate representative core five- and six-membered ring structures of the ER ligands of this invention. The cores include five-member cyclic rings that are doubly unsaturated and which may contain one or two heteroatoms (particularly N, O or S) . The cores also include six-member aromatic rings which may contain one or two heteroatoms (particularly N). The selected cores can accommodate from three to six substituents which can be oriented by placement on ring elements. The representative cores listed are distinct from one another in the position of the R^1 substituent on the selected rings with respect to heteroatoms (and/or double bonds) and other substituents therein, in the cyclopentadienes with respect to the tetrahedral carbon, or in the cyclopentadienones with respect to the C=O group on the ring. Dependent upon the selection of a particular R^1-R^6 or $R^{5'}$ substituent distinct structures may be obtained by interchange of substituents.

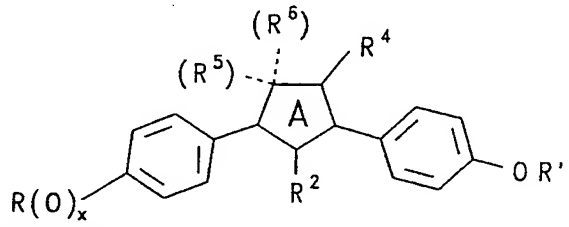
In specific embodiments, ER ligands of this invention can have the structures:



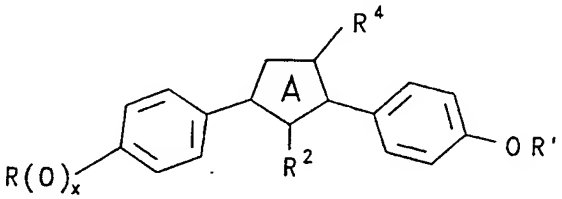
where x is 0 or 1 and R is hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other substituents are defined as in the Summary above;



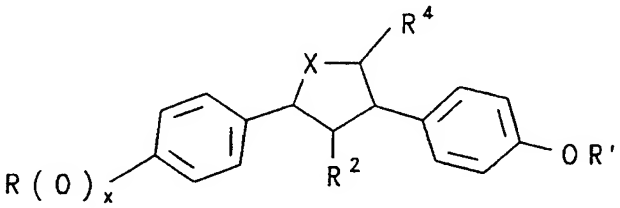
where x is 0 or 1 and R is hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, where R' is a phenyl ring substituent as defined above in the definition of R^3 and can be a polar or basic substituent and other variables are defined as in the Summary above;



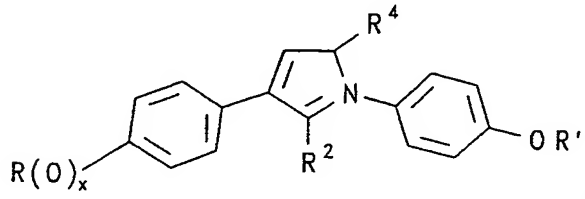
where x is 0 or 1, R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;



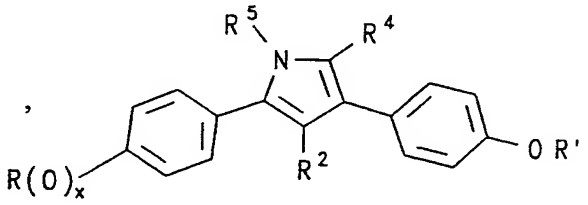
where x is 0 or 1, R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;

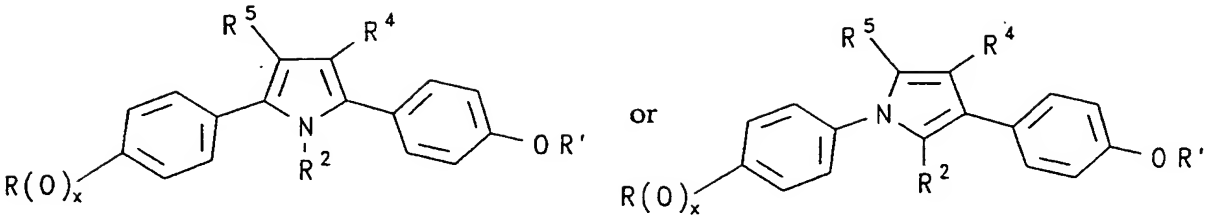


where x is 0 or 1, X is N, NH, NR^5, S or O and R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;

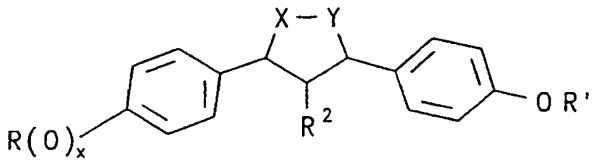


,

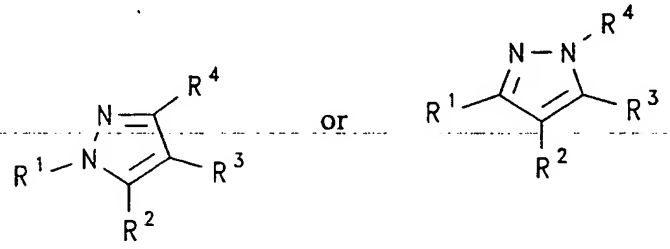




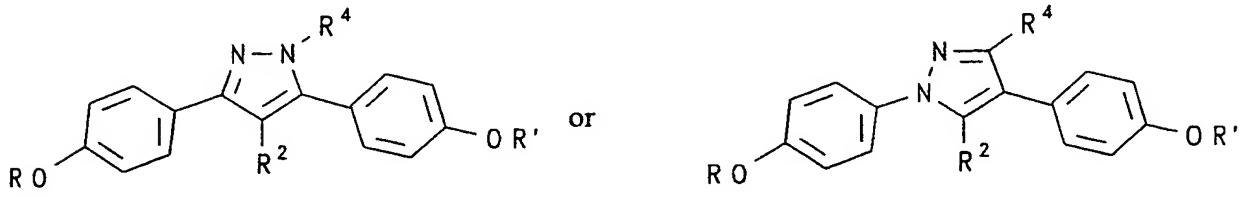
where x is 0 or 1, X is N, NH, NR^5, S or O and R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;



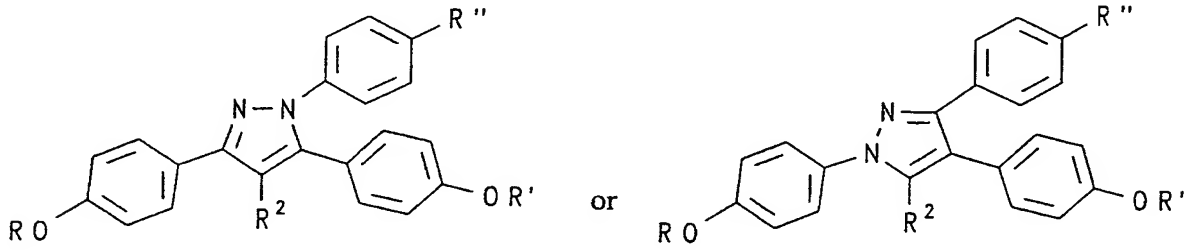
where one of X or Y is N and the other of X or Y is N, S or O, x is 0 or 1 and R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;



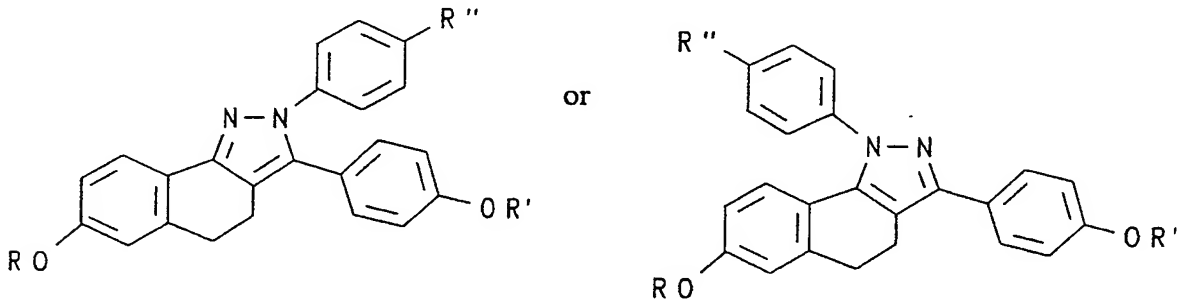
where R^1-R^4 are defined as in the Summary above;



where R^2 and R^4 are defined as in the Summary above and R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group;



where R^2 is defined as in the Summary above, R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and R" can be a hydrogen, a halogen, a hydroxy, an alkoxy, or a basic or polar group; and

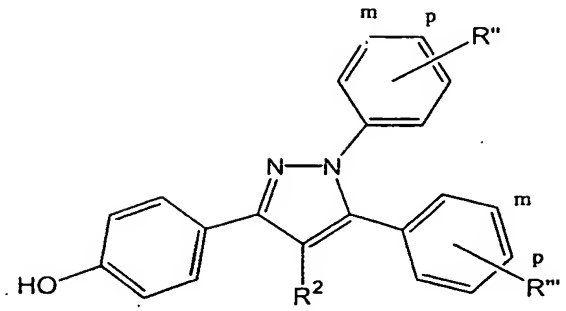


where R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and R" can be a hydrogen, a halogen, a hydroxy, an alkoxy, or a basic or polar group.

Preferred R and R' are H, preferred R" is OH and preferred R^2 are straight-chain or branched lower alkyl groups having up to 6 carbons atoms.

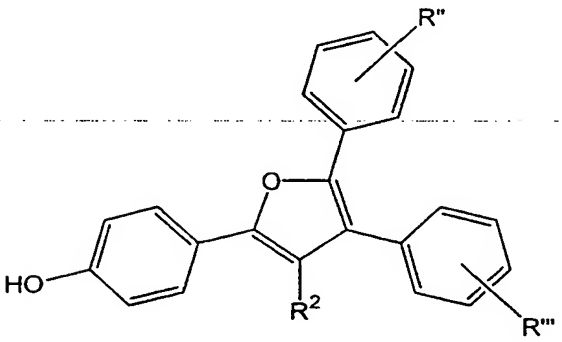
Specific compounds having the illustrated structures are listed in Scheme 18.

Pyrazoles of particular interest having significant ER binding affinity (RBAs) include those having the structure:



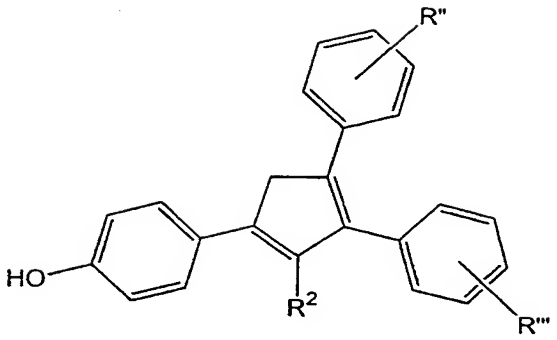
where R^2 is an ethyl, n-propyl, isopropyl, n-butyl, isobutyl or t-butyl group, R″ and R‴ may be positioned at the *meta* or *para* ring positions and can be selected independently of each other from the group p-H, p-OH, p-F, p-Br, p-CH_3, m-OH, m-F, or m-Br. Pyrazoles of this structure exhibiting generally higher ER affinity are those in which R‴ and R″ are both p-OH.

Furans of particular interest having significant ER binding affinity (RBAs) include those having the structure:



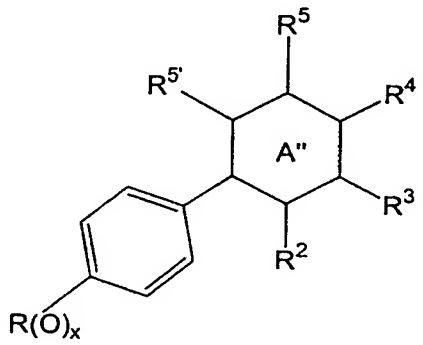
where R^2 is an ethyl, n-propyl, isopropyl, n-butyl, isobutyl or t-butyl group, R″ and R‴ may be positioned at the *meta* or *para* ring positions and can be selected independently of each other from the group p-H, p-OH, p-F, p-Br, p-CH_3, m-OH, m-F, or m-Br. Furans of this structure exhibiting generally higher ER affinity are those in which R‴ and R″ are both p-OH.

Cyclopentadienes of particular interest include those having the structure:

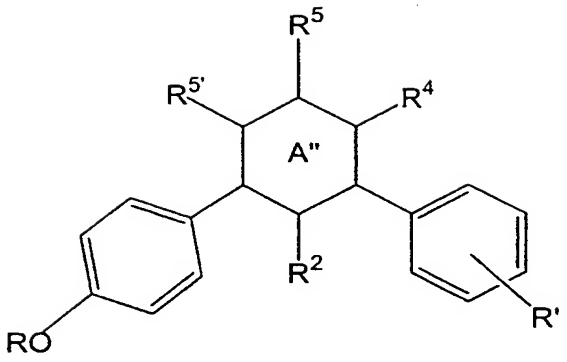


where R^2 is an ethyl, n-propyl, isopropyl, n-butyl, isobutyl or t-butyl group, R″ and R‴ may be positioned at the *meta* or *para* ring positions and can be selected independently of each other from the group p-H, p-OH, p-F, p-Br, p-CH_3, m-OH, m-F, or m-Br. Furans of this structure exhibiting generally higher ER affinity are those in which R‴ and R″ are both p-OH.

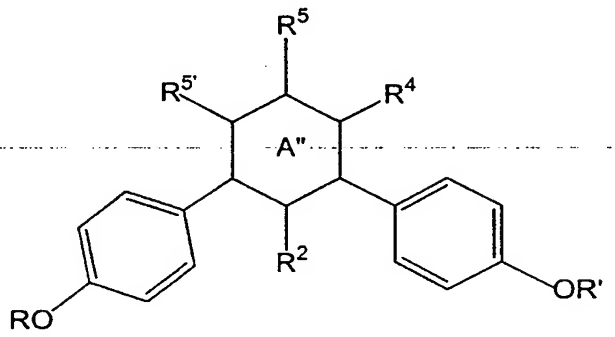
In specific embodiments, ER ligands of this invention can have the structures:



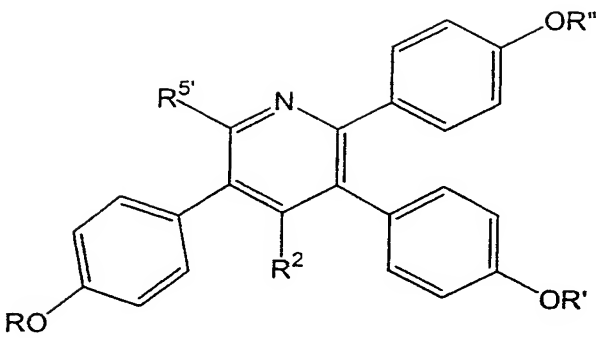
where the A″ is an aromatic ring with up to two heteroatoms in the ring, x is 0 or 1 and R is hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, other substituents are defined as in the Summary above, and one or two of the indicated substituents may be absent due to heteroatom placement;

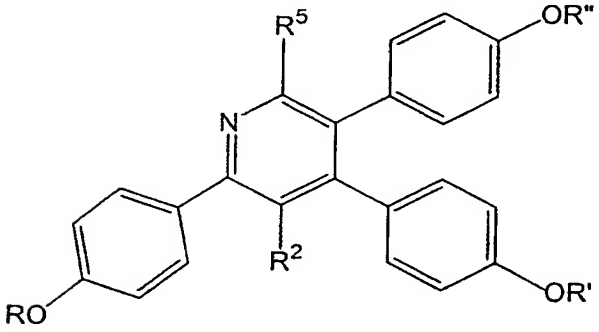


where A″ is an aromatic ring with up to two heteroatoms in the ring, R is hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, R' is a phenyl ring substituent as defined above in the definition of R^3 which may be at any ring position (preferably para or meta ring positions) and can be a polar or basic substituent, other substituents are defined as in the Summary above, and one or two of the indicated substituents may be absent due to heteroatom placement;

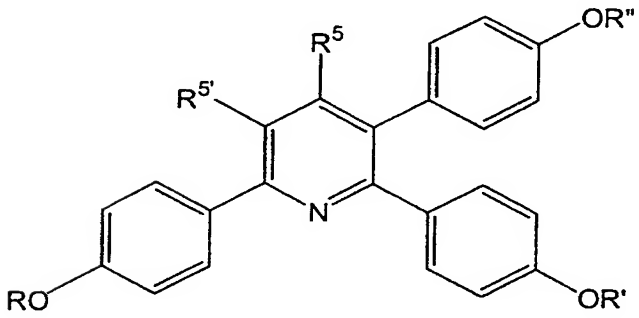


where R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, other variables are defined as in the Summary above and one or two of the indicated substituents may be absent due to heteroatom placement;



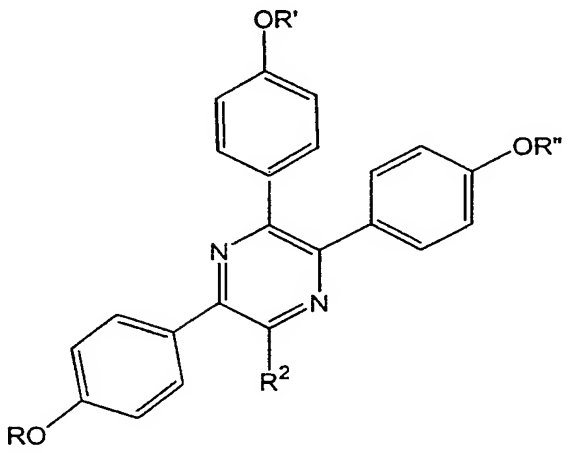


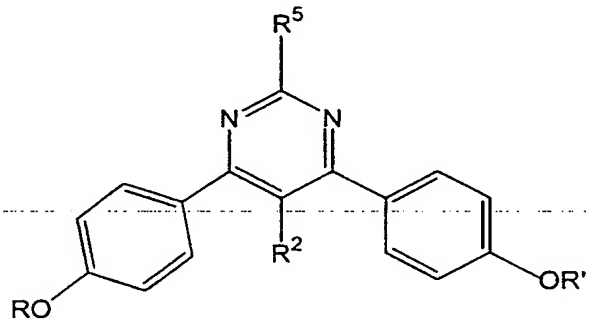
or



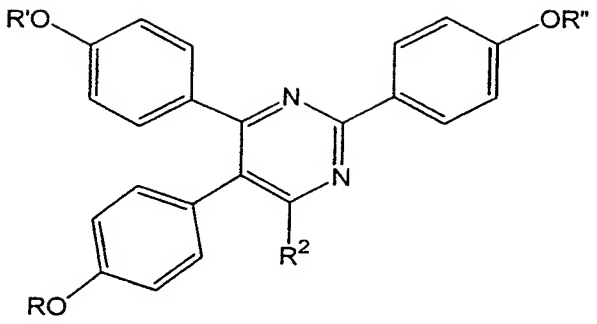
where R, R' and R" can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group and other variables are defined as in the Summary above;

or



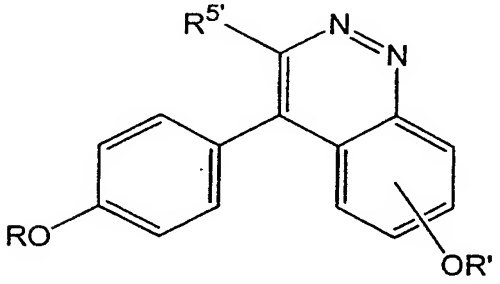


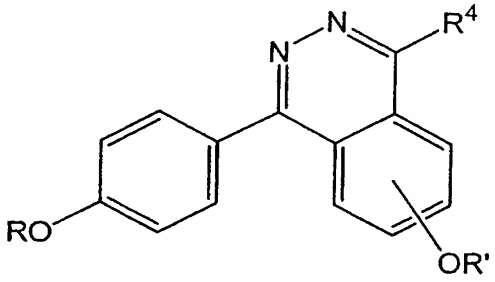
where R, R' and R" can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, other variables are defined as in the Summary above;

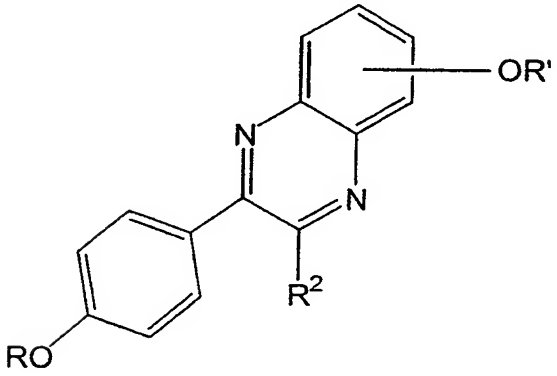


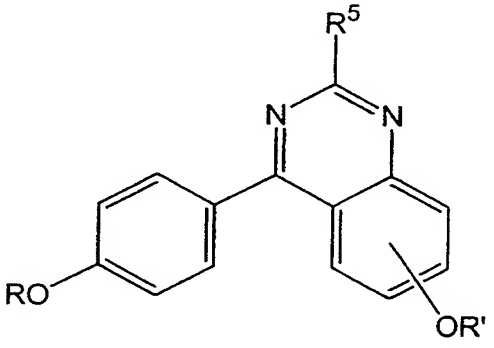
where R, R' and R"can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group where R^2 is defined as in the Summary above, but is preferably a lower alkyl groups having up to about 6 carbon atoms; and

Any of:

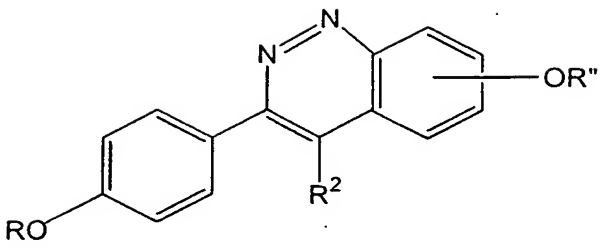






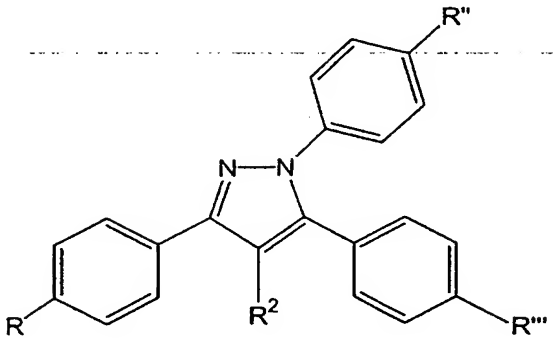


or



where R and R' can be the same or different and can be hydrogen, alkyl, alkenyl, alkynyl or substituted alkyl, alkenyl, alkynyl, or a basic or polar group, other substituents are as described above in the Summary .

Five- and six-membered ring core compounds of this invention substituted with a basic or polar side chain are of particularly interest as potential SEEMS (selective estrogen receptor modifiers) which can display agonist or antagonist activity (or a mixture of these activities) that can vary from tissue to tissue. Basic or polar side chain can be substituted at several possible positions in the compounds of this invention. For example, in pyrazoles of the following structure:



a basic or polar group can be substituted at R, R^2, R″or R‴. A preferred positioning of the basic or polar side chain is such that it would occupy a region of the ligand binding pocket normally occupied by such groups in known SERMS, as well as complete estrogens antagonists. Basic side groups of particular interest for substitution into pyrazoles include those carrying alicyclic amine groups, e.g.,: $-(X)_x(CH_2)_n$-NRR' where X is O or S, x is 0 or 1, n is an integer from 1 to about 10 and preferably 2 to 6, and R and R', can be the same or different and can be alkyl, aryl, or alicyclic. R and R' in these specified basic groups can

together form an heterocyclic or a substituted heterocyclic ring. A preferred basic side group is a piperidinylethoxy group. Preferred R^2 are lower alkyl groups having up to about 6 carbon atoms.

Synthesis of ER Ligands

Imidazoles – The synthesis of representative symmetrical members of the imidazole class (specifically core structure IM1 of Table 1) and their N-alkyl analogs is accomplished by a well known approach [Sarshar, S., Siev, D. & Mjalli, A.M.M. (1996). Imidazole libraries on solid support. *Tetrahedron Lett.* **37**, 835-838] as shown in Scheme 1A. Refluxing 4,4'-dimethoxybenzil **(1)** in formamide in the presence of *para*-formaldehyde affords the 4,5-disubstituted imidazole **2** [Bredereck, H., Gompper, R. & Hayer, D. (1959). Imidazole aus α-Diketonen. *Chem. Ber.* **92**, 338-343], which upon deprotection with BBr_3 in CH_2Cl_2 affords the imidazole **3** in good yield. A similar reaction using 4-methoxybenzaldehyde affords the 2,4,5-trisubstituted imidazole **4** [Lombardino, J.G. & Weisman, E.H. (1974), "Preparation and antiinflammatory activity of some nonacidic trisubstituted imidazoles,". *J. Med. Chem.* **17**, 1182-1188; Schubert, V.H., Giesemann, G., Steffen, P. & Bleichert, J. (1962), "p-Aryl- und p-Alkoxyphenyl-imidazole," *J. Prakt. Chem.* **18**, 192-202; Hayes, J.F., Mitchell, M.B. & Wicks, C. (1994), "A novel synthesis of 2,4,5-triarylimidazoles," *Heterocycles* **38**, 575-585]. To prepare tetrasubstituted systems, the sodium salt of imidazole **4** is alkylated with ethyl, propyl, and butyl iodide, and then deprotected to afford free phenols **6a-d**.

Unsymmetrical, imidazoles (IM1 core) are synthesized as outlined in Schemes 1B and 2. Scheme 1B illustrates the synthetic approach to N-ethyl imidazole **12**. Reaction of 4-methoxy-deoxybenzoin **(7)** [Gardner, P.D. (1956), "Organic peracid oxidation of some enol esters involving rearrangement," *J. Am. Chem. Soc.* **78**, 3421-3424] with bromine and a trace of $AlCl_3$ in Et_2O gives α-bromoketone **8** [Jenkins, S.S. (1934) "The grignard reaction in the synthesis of ketones. IV. A new method of preparing isomeric unsymmetrical benzoins,". *J. Amer. Chem. Soc.* **56**, 682-684] which, upon reaction with sodium azide in acetone, affords the corresponding azide **9**. The azido-ketone **9** is treated with one equivalent of Et_3N and imine **10** in THF. Removal of solvent and excess Et_3N followed by treatment of the crude intermediate 2,5-dihydro-2-hydroxyimidazole with TFA in CH_2Cl_2, according to the procedure of Patonay [Patonay, T. & Hoffman, R.V. (1995), "Base-Promoted Reactions of α-

Azido Ketones with Aldehydes and Ketones: A Novel Entry to α-Azido-β-hydroxy Ketones and 2,5-Dihydro-5-hydroxyoxazoles," *Journal of Organic Chemistry* **60**, 2368-2377], results in the formation of *N*-ethyl imidazole **11**. Deprotection with $BF_3 \cdot SMe_2$ in CH_2Cl_2 produces imidazole **12** in good yield.

The N-aryl substituted imidazoles (IM2 core of Table 1), as exemplified by imidazole **17** can be synthesized as outlined in Scheme 2. Refluxing 4'-methoxy-α-bromobutyrophenone **(13)** with *p*-anisidine in acetone gives the α-amino-ketone **14**, which is converted into the benzamide **15** upon reaction with benzoyl chloride and base. Cyclization with ammonium acetate in refluxing acetic acid affords the 1,2,4,5 tetrasubstituted imidazole **16**, which upon deprotection with $BF_3 \cdot SMe_2$ in CH_2Cl_2 produces the free phenol **17**.

The methods illustrated in Schemes 1A, 1B and 2 can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having imidazole ring core structures. Details of syntheses of representative imidazole are provided in the Examples.

Thiazoles – The synthesis of representative thiazoles is shown in Scheme 3. Thioamide **19** (when R' = Me), derived from 4-alkoxybenzonitrile (**18** when R' = Me) [Taylor, E.C. & Zoltewicz, J.A. (1960), "A new synthesis of aliphatic and aromatic thioamides from nitriles," *J. Am. Chem. Soc.* **82**, 2656-2657] is condensed with 4'-alkoxy-α-bromoacetophenone (**20** when R' = Me) or 4'-alkoxy-α-bromobutyrophenone (**13** when R' = Me) in refluxing DMF to give the 2,4-disubstituted thiazole, e.g., **21a**, [Dolling, K., Zaschke, H. & Schubert, H. (1979), "Kristallin-flussige Thiazole," *J. Prakt. Chem.* **321**, 643-654] or 2,4,5-trisubstituted thiazole, e.g., **21b**, respectively. Deprotection with BBr_3 affords moderate yields of the free phenols, e.g., **22a** and **22b**. Details of a representative syntheses are provided in the Examples.

The methods illustrated in Scheme 3 can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having thiazole ring core structures.

Oxazoles – Representative oxazoles can be synthesized, as outlined in Schemes 4A and B. Reaction of the lithium anion of dithiane **23** with *p*-methoxybenzyl bromide gives the alkylated product (**24** when R = Me) which upon hydrolysis affords 4'-alkoxy-deoxybenzoin (**25** when R = Me) [Katrizky, A.R., Boulton, A.J. & Short, D.J. (1960), "Interaction at a distance in conjugated systems. Part III. Effect of aryl and heteroaryl groups on the infrared intensities of C=C and C+C stretching bands," *J. Chem. Soc.* , 1519-1523] in excellent yield. Conversion to the bromide (**26** when R = Me) [Cowper, R.M. & Stevens, T.S. (1940), "Mechanism of the reaction between arylamines and benzoins," *J. Chem. Soc.* , 347-349] and azide (**27** when R = Me) is accomplished as described for analogous compounds **8** and **9** above. The azido-ketone **27** is then treated with one equivalent of Et_3N and *p*-anisaldehyde, and then with TFA to afford oxazole (**28** when R = Me) [Strzybny, P.P.E., van ES, T. & Backeberg, O.G. (1969), "Reaction of α-acyloxyketones with ammonium acetate," *J. South African Chem. Inst.* **22**, 158-164]. Oxazole **30** results from the condensation of bromo-ketone **26** with *p*-methoxybenzamide in refluxing toluene (Scheme 4B) analogous to the thiazole synthesis discussed above. Deprotection of **28** and **30** with $BF_3 \cdot SMe_2$ gives oxazoles **29** and **31**, respectively. Details of a representative synthesis are provided in the Examples.

The methods illustrated in Schemes 4A and B can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having oxazole ring core structures.

Pyrazoles – The synthesis of the pyrazoles is illustrated in Schemes 5A-C. Scheme 5A involves the condensation of a hydrazine with a 1,3-diketone [Marzinzik, A.L. & Felder, E.R. (1996), "Solid support synthesis of highly functionalized pyrazoles and isoxazoles; scaffolds for molecular diversity," *Tetrahedron Lett.* **37**, 1003-1006]. The method of Beak [Reitz, D.B., Beak, P., Farney, R.F. & Helmick, L.S. (1978), "Dipole-stabilized carbanions from thioesters. Evidence for stabilization by the carbonyl group," *J. Am. Chem. Soc.* **100**, 5428-5436] can be used to obtain 1,3-diketone **33** from the reaction of the methyl thioester **32** and lithium tetramethylpiperidide. Condensation of the diketone with hydrazine hydrochloride or N-substituted hydrazine hydrochlorides in refluxing DMF/THF (1:1) affords the 3,5-disubstituted pyrazole **34a** or 1,3,5-trisubstituted pyrazoles **34b-d**; yields can be

higher with aryl-substituted hydrazines than with hydrazine itself [van Steenis, J. (1946), "The nitration of dianisoylmethane and p-methoxydesoxybenzoin," *Chem. Ber.* 29-46; Hergenrother, P.M. (1991), "New Developments in Thermally Stable Polymers," *Rec. Travl. Chim. Pays-Bas.* **110**, 481-491; Ando, W., Sato, R., Yamashita, M., Akasaka, T. & Miyazaki, H. (1983), "Quenching of singlet oxygen by 1,3,5-triaryl-2-pyraazolines," *J. Org. Chem.* **48**, 542-546]. Deprotection of **34a-d** with BBr_3 affords the free phenols **35a-d**.

The introduction of a 4-alkyl substituent was accomplished through the alkylation of diketone **33** with TBAF and an alkyl iodide (e.g., ethyl iodide) to afford **36**. [Tewari, S.C. & Rastogi, S.N. (1979), "Studies in antifertility agents: Part XXII: 1,2-diethyl-1,3-bis-(p-hydroxyphenyl)-1-propene," *Ind. J. Chem.* **18B**, 62-64; Clark, J.H. & Miller, J.M. (1977), "Hydrogen bonding in organic synthesis. Part 6. C-Alkylation of β-dicarbonyl compounds using tetralkylammonium fluorides," *J. Chem. Soc., Perkin I*, 1743-1745] . Conversion of diketone **36** to the corresponding pyrazoles is accomplished as with the unsubstituted case, to afford pyrazoles **38a-d**. Details of representative syntheses are provided in the Examples.

Alternatively, ketone **90** can be reacted with 2 eq. of nitrobenzyl ester **91** and $LiN(iPr)_2$ to give the 1,3-diketone which is then taken to the pyrazole (e.g., **38**) as further indicated in Scheme 5A (Path B).

Scheme 5B provides more detail of the syntheses of pyrazoles **200-204** via the method of Scheme 5A.

Scheme 5C presents a general method for synthesis of pyrazoles having core PA2 in which R^1 is attached to a ring. This scheme also illustrates a method for addition of I to the ring. Any halogen can be added by appropriate selection of reagent. Scheme 5D provides more detail of the syntheses of pyrazoles **205-209** via the method of Scheme 5C.

The methods illustrated in Schemes 5A-D can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having pyrazole ring core structures.

Isoxazoles – An illustrative preparation of an isoxazole is shown in Scheme 6 [Perkins, M., Beam, C.F., Dyer, M.C.D. & Hauser, C.R. (1988), "3-(4-Chlorophenyl)-5-(4-methoxyphenyl)isoxazole," *Org. Syn. Coll. Vol.* **VI**, 278-281]. Double deprotonation of the ketoxime (**39** when R^2 is H) derived from 4-methoxyacetophenone with n-BuLi, followed by addition of methyl 4-methoxybenzoate affords the 3,5-disubstituted isoxazole e.g., **40** [Ichinose, N., Mizuno, K., Tami, T. & Otsuji, Y. (1988), "A novel NO insertion into cyclopropane ring by use of $NOBF_4$. Formation of 2-isoxazolines," *Chem. Lett.* , 233-236]. Deprotection with BBr_3 afforded the free phenol **41** [Murthy, A.K., Rao, K.S.R.K.M. & Rao, N.V.S. (1968) "Isoxazolylphenols and their absorption spectra," *Aus. J. Chem.* **21**, 2315-2317]. Details of a representative synthesis are provided in the Examples.

The methods illustrated in Scheme 6 can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having isoxazole ring core structures.

Isothiazoles- Illustrative preparations of isothiazoles are shown in Schemes 7A and B. Reaction of the thioketone imine **42** with iodine results in cyclization to form isothiazole **43** (Scheme 7A). Alternatively, isoxazoles (as prepared in Scheme 6) can be reductively cleaved to form enaminoketone **44** which on treatment with P_2S_5 /chloranil results in isothiazole **43**.

The methods illustrated in Schemes 7A and B can be employed or readily adapted using well-known methods and by appropriate choice of starting materials by one of ordinary skill in the art for the synthesis of ER ligands of this invention having isothiazole ring core structures.

Furans, Thiophens and Pyrroles: Heterocycles having one heteroatom in the 5-membered ring core (e.g., furans, thiophenes and pyrroles) can generally be prepared by cyclization of appropriately substituted 1,4-diketones. Ring substitution is for the most part determined by selection of the 1,4-diketone. The synthesis of 1,4-diketones is illustrated in Scheme 8. Starting with aldehydes and ketones that are commercially available or readily synthesized by well-known methods, substituted α, β-unsaturated ketones are formed by

treatment with ethanolic KOH. The α, β-unsaturated ketones are transformed using, for example, the Stetter reaction with appropriately substituted aldehydes in the presence of a thiazolium salt catalyst (e.g., 3-benzyl-5-(2-hydroxyethyl)-4-methyl-thiazolium chloride for aliphatic aldehydes or 3-ethyl-5-(2-hydroxyethyl)-4-methyl-thiazolium bromide for aromatic aldehydes) to form the desired diketones. See: Khanna, I.K. et al. (1997) J. Med. Chem. **40** :1619-1633 and Stetter, H. (1976) Angewandte Chemie Int'l Ed. Eng. **15**: 639-647. Several exemplary diketones **53-56** are listed in Scheme 8. These diketones can be converted into furans (Scheme 8), thiophenes (Scheme 9) or pyrroles (Schemes 10A-D).

Furans- Acid catalyzed cyclization of the diketones, illustrated in Scheme 8 for diketones **53-56** give furans **57-59** in 85-93% yields (Wu, A. et al. (1997) Synthetic Comm. **27**:2087-2091). Also illustrated is addition of a halogen substituent (Y) to the furan ring. The synthesis of additional exemplary furans **210-214** is illustrated in Scheme 8A where a synthesis of 1,4-diketone precursors is illustrated at the top of the scheme.

An alternate approach to 1,4-diones is illustrated in Scheme 8B which uses enolate chemistry employing α-bromoketones as electrophiles. Desoxyanisions were treated with one equivalent of potassium bis(trimethylsilyl) amide followed by addition of α-bromoketone to give the desired tetra-substituted diones in good yield. This approach affords the 1,4-diones as mixtures of diastereomers, however, no separation of the stereoisomers is required, as these centers become non-stereogenic in the final products.

Thiophenes-Treatment of diketones as illustrated in Scheme 9 for diketones **54** and **56** with Lawesson's Reagent gives thiophenes **60** and **61** in about 85% yields [Kiebooms, R.H.L. et al. (1997) J. Org. Chem. **62**:1473-1480]. Also illustrated is addition of a halogen substituent (Y) to the furan ring.

Pyrroles- Acid catalyzed cyclization of diketones in the presence of a selected primary amine as illustrated in Schemes 10A-B and 10D for diketones (such as **53** and **55**) results in the formation of N-substituted pyrroles PR3 (e.g., **62** where $R^1 = R^3 = C_6H_4\text{-}OCH_3$, $R^2 = C_2H_5$ and $R^4 = C_6H_5$) and PR2 [Khanna, I.K. et al. (1997) J. Med. Chem. **40**:1619-1633]. Reaction of the a 1,4-diketone, e.g., **54**, with ammonium acetate in acetic acid results

in a pyrrole PR2 where R^2 is H (such as **64** where $R^1 = R^3 = C_6H_4\text{-}OCH_3$, and $R^4 = C_6H_5$) in Scheme 10C. Deprotonation of the N-H pyrrole with sodium hydride followed by alkyl iodide addition (illustrated for ethyl iodide) gives the N-R^2-substituted analog . Scheme 10C also illustrates a method for introducing a halogen onto the pyrrole ring.

Scheme 10D illustrates a synthesis of a pyrrole of core structure PR1 (with N-R^1). It is apparent from an overview of Schemes 10A-D that a variety of different pyrroles with different relative positions of substituents R^1-R^5 with respect to each other and with respect to the N in the ring can be obtained by appropriate substitution of starting 1,4-diketones.

Furans, thiophene and pyrroles having methoxy substituents on substituted phenyl R groups (e.g. **58, 60** and **62**) can be deprotected with boron tribromide to afford the demethylated products.

The methods illustrated in Schemes 8, 8A, 9, and 10A-D can be employed or readily adapted by one of ordinary skill in the art using well-known techniques and methods for synthesis and with appropriate choice of starting materials for the preparation of the furans, thiophenes and pyrroles of this invention.

Cyclopentadienes and Cyclopentadienones: Scheme 11A (including paths A, B, B' and C) illustrates representative syntheses of cyclopentadienes and cyclopentadienones of this invention. Dieneone **93** produced for example by path A is cyclized to give the cyclic unsaturated ketone **94**. Additional non-hydrogen substituents e.g., R^4 and R^6, can be added to the five-membered ring as indicated in path C to ultimately give various cyclopentadienes (e.g., **95A-B**). The cyclic ketone **94** can be reduced via path B to a cyclopentadienone **96A**. Also, a cyclopentadiene having two hydrogens **97** on the same ring carbon can be oxidized to give a cyclopentadiene **96B**.

Scheme 11B illustrates a synthesis of cyclopentadienones of this invention and an alternative synthesis of cyclopentadienes. A cyclic unsaturated ketone **98** is prepared by cobalt carbonyl catalyzed cyclization of a substituted alkyne and olefin. This reaction can

result in the generation of regioisomers. Ketone **98** is reduced to give cyclopentadiene **97**. Scheme 11C provides another general scheme for synthesis of cyclopentadienones.

Scheme 11D illustrates another alternative synthesis of cyclopentadienes of this invention as applied to cyclopentadiene ligands **230-235** with $R^2 = C_2H_5$. Cyclopentadienyl ligands with R^2 that is a lower alkyl group, e.g., n-propyl, can also be made by this method by selection of Grignard reagent. Compounds **236** and **237** where R^2 is n-propyl can also be made by this method. In this method, a thiazolium salt catalysed addition of an aldehyde to an α, β-unsaturated ketone under Stetter conditions gives the corresponding 1,4-diketone. Intramolecular aldol condensation of the 1,4-diketone using methanolic potassium hydroxide gives cyclopentenones. Cyclopentadienes are derived from Gringard and dehydration reaction on the cyclopentenones. However, the cyclopentadienes were not stable to the conditions of deprotection to release free phenol. So the cyclopentenones were deprotected under mild conditiond (borontrifluoride-dimethylsulfide) to give the cyclopentenones. The free phenols were then temporarily reprotected as their trimethylsilyl ehters using bis(trimethysilyl)acetamide which were then subjected to Gringnard reaction. Dehydration of the tertiary alcohol and removal of the trimethylsilyl group were achieved under acidic work-up conditions following the Grignard reaction to give desired cyclopentadienes. The cyclopentadienes obtained were found to be very sensitive to air, heat and acidic impurities in solvents.

The methods illustrated in Schemes 11A-D can be employed or readily adapted by one of ordinary skill in the art using well-known techniques and methods for synthesis and with appropriate choice of starting materials for the preparation of the cyclopentadienes and cyclopentadienones of this invention.

Base Substituents: Schemes 12A-D illustrate representative methods for introduction of basic amino substituents into five-membered ring ER ligands of this invention. The schemes illustrate the synthesis of a base-substituted pyrazole. Intermediate **100** is reacted with a substituted 1,3 diketone to form the pyrazole. Schemes 12B and 12C illustrate in more detail the synthesis of Scheme 12A for the introduction of a piperidinylalkoxy basic group. Scheme 12B illustrates the introduction of the basic side

chain at a ring nitrogen of a pyrazole. Scheme 12C illustrates the introduction of the basic side chain at C(3) of the pyrazole ring. Scheme 12 D illustrates introduction of a basic side chain on a phenyl substituent on the pyrazole ring.

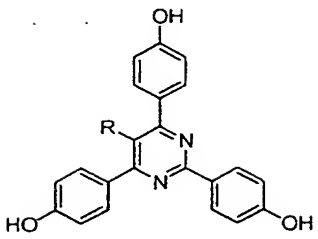
The methods illustrated in Schemes 12A-D can be employed or readily adapted by one of ordinary skill in the art using well-known techniques and methods for synthesis and with appropriate choice of starting materials for the preparation of various amine-substituted ER ligands of this invention.

Six-membered ring heterocycles

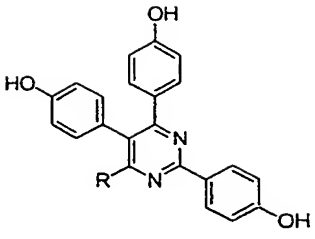
Pyridazine- six-membered ring pyridazine analogues can also be prepared from the 1,4-diones described above for synthesis of furans, thiophenes and pyrroles. As illustated in Scheme 13A, treatment of the diones with hydrazine hydrate followed by air oxidation affords the desired pyridazines. Exemplary pyridazines synthesized by the illustrated methods are indicated in Scheme 13A.

The method illustrated in Scheme 13 A can be employed or readily adapted by one of ordinary skill in the art using well-known techniques and methods for synthesis and with appropriate choice of starting materials for the preparation of the various substituted pyridazines ER ligands.

Pyrimidines- the pyrimidines are classified into two groups (see below) for convenience of description of their synthesis. The first group or Class-I compounds can be prepared as depicted in Scheme 13B. The following numbering applies to Scheme 13B. The reaction of ketone **1** with triflic anhydride in the presence of a nitrile **2** proceeds via a (trifluoromethanesulfonyl)carbenium ion to furnish the pyrimidines **3** in good yields. Deprotection of the phenolic methyl ether in **3** under mild acid conditions leads to the formation of the desired phenol-bearing pyrimidines **4** in good yields.



Pyrimidines : Class-I



Class-II

For the synthesis of Class-II compounds of pyrimidines the synthetic sequence illustrated in Scheme 13C is employed. The following numbering applies to Scheme 13C. However, the use of a ketone **5**, flanked by two methylene groups resulted in the formation of two separable regio-isomeric pyrimidines, obtained by the ring closure at either of the methylene carbons. Deprotection of the phenolic methyl ethers following the same strategy as in Scheme 13A lead to the formation of the regio-isomeric pyrimidines **6** and **7**, respectively. The reaction, when extended to synthesize the pyrimidine **8** (with three phenolic groups), led to the formation of mostly the regio-isomer **9** with only trace amounts of the desired isomer **8**, which in addition was difficult to separate from the excess anisonitrile used in the reaction. Another synthetic procedure that can be applied to the synthesis the desired pyrimidine **8** is illustrated in Scheme 13D. This method can generally be applied to the synthesis of various pyrimidines of this invention.

The methods illustrated in Schemes 13A-D can be employed or readily adapted by one of ordinary skill in the art using well-known techniques and methods for synthesis and with appropriate choice of starting materials for the preparation of a variety of Pyrimidine ER ligands of this invention.

Pyrazines-the synthesis of the pyrazines follows a simple strategy as depicted in Scheme 13E. The following numbering refers to Scheme 13E. Condensation of diketone **10** with the substituted ethylenediamine **11** under acidic conditions furnished the pyrazines **13** in moderate yields. Deprotection of the phenolic methyl ethers of **13** under mild acid conditions furnished in good to excellent yields the pyrazines **13**. By another route, based on the condensation of α–hydroxy ketones with ammonium acetate in ethanol, as depicted in Scheme 13F, a mixture of the pyrazines were synthesized, which on deprotection under

similar conditions as above gave the pyrimidines **13**, **14** (Scheme 13F) and an unseparable mixture of the pyrimidines **15** and **16**(Scheme 13F).

Quinoxalines-the synthesis of the quinoxalines follows a simple strategy as depicted in Scheme14A. The following numbering refers to Scheme 14A. Condensation of the phenylenediamine **1** with α-diketone **2** under acidic conditions furnished in moderate yields a 1:1 mixture of the regio-isomeric quinoxalines **3**. Removal of the phenolic methyl ether using boron triflouride dimethyl sulfide then furnished the deprotected quinoxalines **4** in good yields.

Quinazolines:-the synthesis of the quinazolines can be carried out using the strategy described in Scheme 14B.

Cinnolines-the synthesis of cinnolines can be carried out using the strategy described in Scheme 14C.

Phthalazines: the synthesis of phthalazines can be carried out using the strategy described in Scheme 14D.

Combinatorial Methods

Combinatorial chemistry can be employed to synthesize a variety of potential ER ligands having the 5-member and 6-membered unsaturated ring core structures described herein. These solid phase methods allow the production of a combinatorial library of compounds, having varying substituents on the core structure, to test for ER binding and activity. Schemes 15 A and B provides illustrative solid support syntheses of compounds having a heterocyclic ring structure, pyrazoles. These schemes exemplify the use of a resin P, e.g., the Merrifield resin, to tether a starting material. The synthesis proceed on the resin-tethered species and after formation of the desired substituted ring structure, it is released from the resin (i.e., solid support).

Scheme 15A illustrates distinct syntheses for compounds where R^3 is aliphatic (path A) or aryl (path B). This scheme can be used to generate pyrazoles with three or four

substituents. Scheme 15B illustrates an alternate route to pyrazoles proceeding through a distinct intermediate **119** to a tethered pyrazole **109**. Path A in Scheme 15B illustrates halogen addition to the ring, e.g., **123**. The choice of paths in B depends on whether substituent R^2 is aliphatic or aryl.

Scheme 16 provides illustrative solid support syntheses of compounds having a heterocyclic ring structure, oxazoles, thiazoles and imidazoles. Interestingly, a single intermediate **134** in Scheme 16 can be used to generate compounds of all three ring structures **145**, **147**, or **149**.

Estrogen Receptor Binding

ER ligands are those compounds which exhibit measurable binding affinity for the estrogen receptor. There are various ways to measure and quantify ER binding affinity. In this invention ER binding affinity is measured in competitive binding assays compared to estradiol. Binding affinity is expressed as a relative binding affinity (RBA) in percent compared to estradiol which is assigned an affinity of 100%. Substantial affinity for ER is indicated by an RBA of about 0.1% or more. Good affinity binding to ER is indicated by an RBA of about 1%- to about 10%. High affinity binding to ER is indicated by an RBA of about 10% or higher.

The binding affinities of substituted compounds of heterocyclic cores structures listed in Table 1 are shown in Tables 3–5A-B, organized according to heterocyclic core structure. The binding values were obtained from a competitive radiometric binding assay, using [^{3}H]estradiol as the tracer and dextran-coated charcoal to adsorb free tracer or hydroxyapatite to adsorb the ER-tracer complex; the values are expressed as relative binding affinities (RBA), in percent, with respect to estradiol assuming an affinity of 100% for estradiol. Lamb and/or rat uterine cytosol ER preparations were used as described in Katzenellenbogen, J.A. et al. (1977) "Estrogen photoaffinity labels. 1. Chemical and radiochemical synthesis of hexestrol diazoketone and azide derivatives; photochemical studies in solution." Biochemistry **16**:1964-1970.

In some cases, a mixture of regioisomers were prepared and the binding affinity of the mixture was assessed. In particular in the unsymmetrical pyrazole cases (R1 ≠ R3), isomers were formed. In the cases so far studied, the isomers have been found to be formed in comparable amounts, so that the ratios of isomers in the mixtures are likely to be between 2:1 and 1:2. In some cases, the mixtures have been separated and the individual isomers have been tested for binding as pure compounds and significant differences in binding affinity have been found. In most cases, it has not yet been determined which regioisomeric structure corresponds to which separated regioisomer.

If the isomer ratios are within 1:2 or 2:1, then any binding affinity measured for a mixture could never be less than one-third the affinity of the pure high affinity isomer. In the worst case, if one isomer were inactive, and the other active isomer were present as the 1 part in a 1:2 mixture, then when the high binding isomer was pure, it concentration in the binding assay would be 3-fold higher and its measured affinity also 3-fold higher than in the 1:2 mixture. This means that in cases where mixtures have been examined for binding affinity, that one of the isomers present may have up to a 3-fold higher binding affinity than indicated by the measurement.

Without wishing to be bound thereby, the following analysis of the ER binding affinities of individual compounds is provided:

Imidzoles, Oxazoles and Thiazoles – The receptor binding data for several imidazoles are shown in Table 3. Although the members of this series have rather low affinity, there is an increase in RBA with the addition of alkyl substituents at the 1-position (**6a-d**); this trend reaches a maximum for propyl **6c**, reversing for the butyl substituent **6d**. Such trends are well known both in steroidal systems (11β- and 16α-substituents) [Anstead, G.M., Carlson, K.E. & Katzenellenbogen, J.A. (1997). The estradiol pharmacophore: ligand structure-estrogen receptor binding affinity relationships and a model for the receptor binding site. *Steroids* **62**, 268-303], as well as in other non-steroidal ligand series (such as 2-phenylindoles [von Angerer, E., Prekajac, J. & Strohmeier, J. (1984). 2-Phenylindoles. Relationship between structure, estrogen receptor affinity, and mammary tumor inhibiting activity in the rat. *J. Med. Chem.* **27**, 1439-1447], tetrahydrochrysenes [Hwang, K.J., O'Neil, J.P. &

Katzenellenbogen, J.A. (1992). 5,6,11,12-Tetrahydrochrysenes: Synthesis of rigid stilbene systems designed to be fluorescent ligands for the estrogen receptor. *J. Org. Chem.* **57**, 1262-1271] etc.), and probably represent the filling of a preformed pocket of limited volume in the receptor by this substituent [Anstead, G.M., Carlson, K.E. & Katzenellenbogen, J.A. (1997) supra]. The principal difference in binding, however, is between the tetra-substituted imidazoles (**6b-d**, **12**, **17**) and the di- or tri-substituted imidazole (**3** and **6a**), the tetrasubstituted ones having much higher affinity. There is little difference in binding between imidazoles **12** and **17**, which have a different arrangement of nitrogen atoms in the heterocyclic core, but display their four substituents in an identical fashion. The overall low binding affinity of the imidazoles as a class might arise from the high inherent polarity of this heterocyclic system. The dipole moment for imidazole is very large, 5.56 D, and this may be unfavorable for binding to the estrogen receptor.

Table 4 shows the binding data for two thiazoles and oxazoles prepared. Although affinities are again very low, the more highly substituted thiazole again has the higher affinity (**22a** vs. **22b**). The oxazole **29** has undetectable affinity for ER. The isomer **31**, however, does have measurable though low binding. In contrast to imidazoles, thiazoles and oxazoles do not have very high dipole moments; so overall polarity is not likely to be the source of their low ER binding affinity, although heteroatom orientation appears to play a role (**29** vs. **31**). However, in the imidazole series, the compounds with the highest affinities were all tetrasubstituted. Since it is only possible to trisubstitute a thiazole or oxazole, this core structure may be unable to present sufficient peripheral substituents to afford ligands with good ER binding affinities.

The low binding affinities of the imidazoles, thiazoles and oxazoles may be, at least in part, due to their overall structure which is expected to be rather planar. It has been reported that good ligands for the estrogen receptor need to have some degree of "thickness" in the central portion of the ligand [41]. When alkyl substituents are added to either the imidazoles or thiazoles, their RBA increases. This increased binding could be due to an increase in steric bulk around the central portion of the molecule, the result, in part, of a twisting of some of the aromatic substituents (see below) or to an increase in lipophilicity.

Durani et al. (1989) "Structure-activity relationship of antiestrogens: a study using triarylbutenone, benzofuran, and triarylfuran analogues as models for triarylethylenes and triarylpropenones" *J. Med. Chem.* 32:1700-1707 reported receptor affinity and biological activity data for several structural classes including triarylfurans.

Furans- RBA data and differential binding affinities for ERα and ERβ for several furans are given in Table 5 Furan **204**, for example, exhibits relatively high RBA. Several furans exhibit significant binding strength preference for ERα compared to ERβ. Furan **203**, for example, binds to ERα about 70-fold more strongly than it does to ERβ.

Pyrazoles and Isoxazoles – The RBA data for the 1,2-azoles are presented in Tables 6A-B. Immediately apparent is the relatively high binding affinity of pyrazoles **38b** and **38d**. An interesting comparison can be made among compounds **35a**, **35b**, **38a**, and **38b**. The disubstituted progenitor **35a** has very low affinity; addition of a third substituent, 1-phenyl in **35b** or 4-ethyl in **38b**, causes only a 2-fold or 3-fold increase in binding affinity, respectively. By contrast, addition of the fourth substituent (to give **38b**) causes either an 800- or 500-fold increase in binding affinity, respectively. Clearly, this is not additive behavior – two groups that each alone raise binding affinity 2- and 3-fold, together raise binding not 6-fold but 1500-fold. This suggests that high binding affinity is achieved when there is a detailed and proper match between the peripheral substituents and several subsites on the receptor. In the azole system, it appears that enhanced binding is associated with a tetrasubstituted ring. Consistent with this is the lower affinity of the isoxazole **41**, whose affinity is similar to the most closely related trisubstituted pyrazole **38a**.

There are other interesting trends in the pyrazole series: Replacement of the N-phenyl substituent (**38b**) with an N-benzyl group (**38c**) causes a significant 100-fold reduction in binding. Both of these compounds are tetrasubstituted pyrazoles. The decrease in binding affinity in **38b** vs. **38c** again suggests the need for a detailed match between ligand substituents and receptor subsites: the extra "kink" in the benzyl substituent in **38c** may be repositioning the peripheral substituents in a less favorable geometry. The addition of a hydroxyl group at the para position of the N-phenyl substituent (compound **38d** vs. **38b**) has

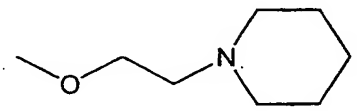
only a minor effect on binding, indicating that polarity is well tolerated in this region of the receptor.

Differential ligand binding affinities for ERα and ERβ can be measured using purified preparations of human ERα and ERβ as described in Example 1B. Using this assay, Pyrazole compound **38b** was found to bind to ERα three-fold more strongly than to ERβ. This result indicates that certain ER ligands of this invention can exhibit differential ligand binding affinity to the different ER subtypes.

ER binding affinities of pyrazole isomers of core structure PA2 (Table 1) are given in Table 7. Overall, these pyrazole isomers have lower ER affinity (RBA) compared to pyrazoles of core structure PA1 by an average of about 2-fold. However, the structure-binding affinity pattern for both pyrazole isomers is quite similar. It is believed that these two core structure pyrazole isomers are binding in the same orientation in the ER binding pocket. Thus, it is possible to permute the position of heteroatoms in the azole ring without major effect on ER binding affinity provided that the peripheral substituents remain disposed with the same geometry and provided that one remains in the same azole series. The pyrazole isomers are compounds with equivalent dipole moment and polarities.

Pyrazoles with basic side groups

Table 8 presents ER binding affinity data for pyrazole of the indicated formula where one of R, R^2, R″or R‴is a cyclic amine group, i.e., a piperidinylethoxy group:



and otherwise R^2 is ethyl, and R is OH, R″and R‴are H or OH groups, as shown in Table 8. The presence of this basic side group has been associated with mixed agonist/antagonist activity. As indicated in Table 8, the pyrazole 301 in which the basic group is substituted at R‴ (with R^2 = ethyl, R and R″ both = OH) gives a very high affinity ER ligand.

RBA of compounds of structures disclosed herein as potential ER ligands either prepared by solution methods or preferably prepared by combinatorial synthetic methods can be readily determined using testing methods disclosed herein. Differential binding affinity of compounds herein can also be readily determined using methods described herein.

Cyclopentadienes- The relative ER binding affinity data of cyclopentadienes **230-237** are provided in Table 9. The ER binding affinities of the cyclopentadiene ligands are generally lower than those of pyrazoles, but exhibit similar patterns of binding affinity as a function of substituents. Cyclopentadiene **235** exhibits relative high ER binding affinity of 8.91%.

Pyridazines- All pyridazines (see, Table 13A) that have been assessed for binding to ER have exhibited no measurable binding affinity. The pyridazines are much more polar than the other 5- and 6-member ring compounds. It is believed that the high polarity of the core is detrimental to ligand binding to ER.

Pyrimidines-RBA values for several pyrimidines of structure PM4 are provided in Table 10. The binding affinities of these pyrimidines for ER are generally lower than those of the 5-membered ring ligands. Again, however, the compounds exhibit similar patterns of binding affinity as a function of substituents. In addition, pyrimidines of structure PM4 where R^2 = methyl, R^1 and R^5 = pOH-phenyl and R^3 = -CH_2-C_6H_4 or R^3 = -CH_2-C_6H_4-pOH exhibited relatively low RBA of 0.032% and 0.013% respectively. The pyrimidine in which R3 = -CH_2-C_6H_4 exhibited no clear preference for binding to an ER subunit (ERα = 1.26% and ERβ = 0.696%). The pyrimidine in which R^3 = -CH_2-C_6H_4-pOH exhibited a preference for binding to ERα (ERα = 0.417% and ERβ = 0.076%).

A pyrimidine that can be characterized as having structure PM3 where R^1 and R^3 are pOH-phenyl groups, R^4 is a phenyl group and R^5 is ethyl exhibited a reasonable RBA of 1.00% with a clear preference for binding to ERα with ERα = 9.5% and ERβ = 3.24%.

Pyrazines- A mixture of two pyrazines regioisomers of structure PZ1 was assessed for relative ER binding affinity. The isomers where those where R^2 was ethyl, R^1 was pOH-

phenyl and either R^4 or R^5 was p-OH-phenyl. RBA of 2.63% was measured for this mixture and a apparent preference for binding to ERα was observed (ERα = 7.94% and ERβ = 2.24%). A pyrazine of the same structure with R^2 = ethyl, and all of R^1, R^4 and R^5 = p-OH-phenyl was found to have a significantly lower RBA of 0.263%. This pyrazine exhibited a similar apparent preference for for binding to ERα with ERα = 7.41% and ERβ = 2.51%.

Quinoxalines- two mixtures containing quinoxaline regioisomers of structure QX2 (Table 2) were assessed for ER relative binding affinity (RBA) and binding affinity to the individual ER subunits. The pair of regioisomers, where R^2 = ethyl, R^1 = p-OH-phenyl with either R' = OH or R" = OH, exhibited a relatively low RBA of 0.20% with an apparent preference for binding to ERβ (ERα = 0.537% and ERβ = 0.933%). The pair of regioisomers, where R^2 = n-propyl, R^1 = p-OH-phenyl with either R' = OH or R" = OH, exhibited a much lower RBA of 0.014% and again exhibited a clear preference for binding to ERβ (ERα = 0.03% and ERβ = 0.224%).

Agonist/Antagonist Character of ER Ligands

Compounds are tested as ER agonists/antagonists in transcriptional activation assays in cells expressing ERα or ERβ. Cells are transfected with an expression plasmid for ERα or ERβ together with an estrogen-responsive reporter gene construct e.g., $(ERE)_3$-pS2-CAT, and treated with increasing concentrations of the test compound or with estradiol for comparison. Reporter gene expression is a measure of the capacity of ER complexed with various compounds to activate transcription, and it is followed as a function of concentration of the test compound. Potency and agonist character in activating transcription is measured relative to activation of the same system by estradiol. The ability of the test compound to inhibit transcriptional activation by increasing concentrations of estradiol is also measured as a function of test compound concentration. The ability of a test compound to inhibit transcriptional activation by estradiol is a measure of antagonist character and antagonist potency of the test compound.

Transcriptional activation can be assessed with ERα or ERβ and in different cells types. Using the $(ERE)_3$-pS2-CAT reporter, CAT activity is measured as a function of the concentration of added test compound (typically ranging from 10^{-12} - 10^{-6} molar) in the

presence or absence of the known stimulator (estradiol, typically ranging from 10^{-12} - 10^{-6} molar). Agonist and/or antagonist character can be selective for ERα and ERβ. Assays can be performed, for example, in human endometrial cancer (HEC-1) cells, Chinese hamster ovarian (CHO) cells, and HeLa cells. Agonist/antagonist character can also be assessed with various promoters, e.g., the estrogen-responsive pS2 promoter, the simple TATA promoter, a non-consensus lactoferrin estrogen-responsive promoter, a heterologous thymidine kinase promoter and the complement C3 promoter which is an estrogen-responsive promoter that contains a non-consensus ERE.

The agonist/antagonist character of a given test compound relative to a selected ER ligand, e.g., estradiol, can be assessed using the transcriptional activation assays described. A given compound may be a pure agonist activating expression and exhibiting no transcriptional inhibition, a pure antagonist suppressing stimulation of expression by known activators and not stimulating transcription themselves or a mixed agonist/antagonist showing both types of behavior. Test compounds may exhibit selectivity in potency, where a given test compound stimulates transcription at lower concentration through one ER subtype than through the other ER subtype. Test compounds may exhibit selectivity in that they stimulate transcription or inhibit expression to a greater degree through one or the other of ERα and ERβ. Test compounds can exhibit a different level of potency for activation compared to inhibition of stimulation of gene expression.

Figures 1A and B are graphs of transcriptional activation by ERα and ERβ, respectively, i response to pyrazole compound **38b** in HEC-1 cells using $(ERE)_3$-pS2-CAT. The figures plot CAT reporter activity as a function of the concentration of the ER ligand. Both figures also show the effect of estradiol (E2) on transcriptional activation by the ER subunits. Pyrazole **38b** is an ERα potency selective agonist compared to estradiol. The pyrazole exhibited a 120-fold higher potency in activating transcription via ERα than via ERβ. In contrast, estradiol exhibits significantly lower activation selectivity between ERα and ERβ. Similar ERα potency-selective character was observed for this pyrazole in other cell types and with other estrogen-responsive promoters. As noted above pyrazole compound **38b** was found to bind to ERα three-fold more strongly than to ERβ. Thus, differences in relative binding of the ligand does not fully account for the significantly higher (120-fold)

selectivity for activation exhibited by the pyrazole with ERα compared that exhibited by the pyrazole with ERβ. These results suggest that factors beyond ligand-receptor interaction, such as receptor-coactivator interactions are likely important determinants of transcriptional potency.

Figure 2 is a graph of transcriptional activation by ERα (diamonds) and ERβ.(squares) in response to pyrazole 334 and pyrazole 336. Both of the pyrazoles assayed are potent in activating transcription under the assay conditions through ERα, but are weak or very weak transcriptional activators through ERβ. Both of these pyrazoles are ERα-potency selective agonists. Pyrazole 336 exhibits no activation through ERβ, even at the highest concentrations used. This pyrazole can be classified as an ERα-specific agonist. For both pyrazoles tested, the difference in ERα and ERβ binding affinities parallels the observed potency selectivity or specificity.

The transcriptional activity profiles of pyrazole 301, which carries a basic piperidinylethoxy group, were examined for ERα and ERβ, respectively in HEC-1 cells as described in the Examples. Figures 3A and 3B are graphs of the transcriptional profiles (CAT activity) of pyrazole 301 for ERα and ERβ, respectively. Pyrazole 301 displayed no agonist activity on ERβ (Fig. 3B). However, on ERα this compound was a partial agonist, reaching an efficacy level nearly half that of estradiol at 1 nM (Fig. 3A). Interestingly, as the concentration of compound 301 increases, the ERα agonist activity returns to near basal levels. At high concentrations, pyrazole 301 acts as an antagonist through both ERα and ERβ., its potency as an antagonist through ERα being about 10-fold higher than through ERβ, which is consistent with its higher affinity for the ERα subtype (see Table 8). Pyrazole 301 is unusual, however, in that it exhibits a biphasic agonist-antagonist dose response through ERα. Many compounds exhibit partial agonist activity on ERα, and they are often more complete antagonists on ERβ than on ERα. However, typically, as the concentration of ligand increases, a constant level of efficacy is reached in assays of agonist and antagonist activity. Pyrazole 301, in contrast, demonstrates agonist activity up to nearly 50% that of estradiol, but its efficacy then decreases to only 10%.

The agonist character and antagonist character of compounds of structures disclosed herein as potential ER ligands either prepared by solution methods or preferably prepared by combinatorial synthetic methods can be readily determined using testing methods disclosed herein.

Pharmaceutical Compositions and Methods

This invention is also directed to pharmaceutically acceptable esters and salts of the ER ligands of various formulas and structures disclosed herein. Acid addition salts are prepared by contacting compounds having appropriate basic groups therein with an acid whose anion is generally considered suitable for human or animal consumption. Pharmacologically acceptable acid addition salts include, but are not limited, to the hydrochloride, hydrobromide, hydroiodide, sulfate, phosphate, acetate, propionate, lactate, maleate, malate, succinate, and tartrate salts. All of these salts can be prepared by conventional means by reacting, for example, the selected acid with the selected basic compound. Base addition salts are analogously prepared by contacting compounds having appropriate acidic groups therein with a base whose cation is generally considered to be suitable for human or animal consumption. Pharmacologically acceptable base addition salts, include but are not limited to ammonium, amine and amide salts.

Pharmaceutically acceptable esters of compounds of this invention are prepared by conventional methods, for example by reaction with selected acids. Pharmaceutically acceptable esters include but are not limited to carboxylic acid esters RCOO-D (where D is a cationic form of a compound of this invention and where R is H, alkyl or aryl groups).

This invention is also directed to prodrugs and derivatives which on being metabolized will result in any of the ER ligands of this invention. For example, alkoxy or acetate groups can be metabolized to hydrogens. Labile substituents may be protected employing conventional and pharmaceutically acceptable protecting groups removable on metabolism. Pharmaceutically active compounds may be derivatized by conventional methods to provide for extended metabolic half-life, to enhance solubility in a given carrier, to provide for or facilitate slow-release or timed-release or enhance or affect other drug delivery properties.

Pharmaceutical compositions according to the present invention comprise one or more ER ligands of this invention in association with a pharmaceutically acceptable carrier or exipient adapted for use in human or veterinary medicine. The carrier is generally selected, as is known in the art for the particular application and should be compatible with the active ingredients. Such compositions may be prepared for use in conventional manner in admixture with one or more physiologically acceptable carriers or exipient. The compositions may optionally further contain one or more other therapeutic agents which may, if desired, be known ER ligands (agonists, antagonists and/or mixed agonist-antagonist as appropriate). ER ligands are present in these pharmaceutical compositions in an amount or in a combined amount sufficient to elicit a measurable positive effect on a symptom or condition associated with an estrogen-dependent disorder on administration to an individual suffering from the symptom or disorder.

The ER ligands according to the invention may be formulated for oral, buccal, parenteral, topical or rectal administration. In particular, the ER ligands according to the invention may be formulated for injection or for infusion and may be presented in unit dose form in ampules or in multidose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use. Aqueous vehicles can be provided with pH control agents, electrolyte control or agents that enhance solubility of the active ingredients in the vehicle.

The pharmaceutical compositions according to the invention may also contain other active ingredients such as antimicrobial agents, or preservatives.

In general, pharmaceutical compositions of this invention can contain from 0.001-99% (by weight) of one or more of the ER ligands disclosed herein. ER ligands may be provided as pure regioisomers or as a mixture of regioisomers. Analogously, ER ligands may be provided as a mixture of enantiomeric forms or as a purified enantiomer.

The invention further provides a process for preparing a pharmaceutical composition which comprises bringing a ER ligand of the invention into association with a pharmaceutically acceptable exipient or carrier. The carrier or exipient being selected as is known in the art for compatibility with the desired means of administration, for compatibility with the selected ER ligands and to minimize detrimental effects to the patient.

For administration by injection or infusion, the daily dosage as employed for treatment of an adult human of approximately 70 kg body weight will range from 0.2 mg to 10 mg, preferably 0.5 to 5 mg, which can be administered in 1 to 4 doses, for example, depending on the route of administration and the clinical condition of the patient. These formulations also include formulations in dosage units. This means that the formulations are present in the form of a discrete pharmaceutical unit, for example, as tablets, dragees, capsules, caplets, pills, suppositories or ampules. The active compound content of each unit is a fraction or a multiple of an individual dose. The dosage units can contain, for example, 1, 2, 3 or 4 individual doses for 1/2, 1/3 or 1/4 of an individual dose. An individual dose preferably contains the amount of active compound which is given in one administration and which usually corresponds to a whole, one half, one third or one quarter of a daily dose.

The magnitude of a prophylactic or therapeutic dose of a particular compound will, of course, vary with the nature of the severity of the condition to be treated, the particular ER ligand compound and its route of administration. It will also vary according to the age, weight and response of the individual patient.

The compounds of the present invention are preferably formulated prior to administration. The present pharmaceutical formulations are prepared by known procedures using well-known and readily available ingredients. In making the compositions of the present invention, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material which acts as a vehicle, exipient or medium for the active ingredient. The compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium),

ointments containing for example up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders.

Some examples of suitable carriers, exipient, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.5 to about 150 mg, more usually about 0.1 to about 10 mg, of the active ingredient. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

As a pH adjusting reagent for preparing the pharmaceutical composition, any allowed for preparing medicines can be used, including but not limited to hydrochloric acid-sodium hydroxide, acetic acid-sodium acetate, glycine-sodium chloride-hydrochloric acid, potassium dihydrogenphosphate-disodium hydrogenphosphate, potassium hydrogenphthalate-sodium hydroxide, sodium secondary citrate-hydrochloric acid, sodium dihydrogen-phosphate-disodium hydrogenphosphate, sodium dihydrogenphosphate-dipotassium hydrogen-phosphate, potassium dihydrogenphosphate-dipotassium hydrogenphosphate, tartaric acid-sodium tartrate, lactic acid-sodium lactate, sodium barbital-sodium acetate-hydrochloric acid, succinic acid-boric acid, potassium primary citrate-sodium hydroxide, sodium primary citrate-borax, disodium hydrogenphosphate-citric acid, sodium acetate-hydrochloric acid, glutamic acid-sodium hydroxide, and aspartic acid-sodium hydroxide. Among them, hydrochloric acid-sodium hydroxide, acetic acid-sodium acetate,

glycine-sodium chloride-hydrochloric acid, tartaric acid-sodium tartrate, lactic acid-sodium lactate, sodium acetate-hydrochloric acid, glutamic acid-sodium hydroxide, and aspartic acid-sodium hydroxide.

This invention is further directed to therapeutic methods employing the ER ligands of this invention and pharmaceutical compositions containing them in the treatment of estrogen-dependent or estrogen-related disorders. These methods comprise a step of administering to a patient having the disorder or symptoms thereof a pharmaceutical composition comprising one or a mixture of the ER ligands of this invention where the ER ligand or mixture of ligands is present in the composition at a level or a combined level sufficient to effect a positive biological response. The present invention provides ER ligands that can be used in place of or in combination with currently known pharmaceuticals active in estrogen-dependent or estrogen-related disorders. Certain ER ligands of this invention and certain ER ligands identified by the combinatorial synthetic methods and selective assays described herein can exhibit improved properties (enhanced activity and/or decreased undesired side-effects) for treatment of estrogen-dependent and estrogen-responsive disorders.

The ER ligands of this invention are useful in vitro and/or in vivo for selective activation or repression of expression, dependent upon the agonist or antagonist nature of the ligand or its potency, of a gene regulated by ER. Gene activation or repression can be selective with respect to subtype of ER (e.g., ERα or ERβ), or variant of ER (e.g., splice variant forms, truncated or processed forms, covalently modified forms, etc.).

The ER ligands of this invention are also useful in vitro and/or in vivo for selective regulation of cellular activities under the control of ER. Cellular activities may be regulated in a variety of ways by ER, subtypes of ER or variants of ER, e.g., up or down regulation of a given cellular process. Regulation is selective with respect to subtype of ER (e.g., ERα or ERβ), or variant of ER (e.g., splice variant forms, truncated or processed forms, covalently modified forms, etc.). Cellular activities that may be regulated include both genomic (related to gene expression) or non-genomic activities (not directly related to gene expression, e.g., such as regulation of calcium flux, particularly in bone cells, hormone release, particularly prolactin release from pituitary cells, etc.).

The subtype-selective ER ligands of this invention can also be of general use in the investigation of ER and its functions. These ligands can be employed to better understand structure and conformation of ER (both subtypes) and to elucidate how ER subtypes interact with other molecules and to relate structure, conformation and interaction with other molecules to ER function.

Agents that can act selectively to stimulate or inhibit estrogen action through the individual ER subtypes can be useful in achieving selective regulation of specific responses and specific tissues. For example, ERβ appears responsible for mediating the beneficial effects of estrogens in suppressing vascular cell overgrowth in response to blood vessel injury. Therefore, an ER ligand that antagonizes only ERβ-mediated responses should block this response without blocking desired responses to estrogens that are mediated by ERα, such as maintenance of a favorable profile of blood lipids. Preferred ER ligands of this invention which exhibit selective interaction with ER subtypes can be employed to selectively stimulate or inhibit estrogen action. References that relate to tissue distribution of ER subtypes include: Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson J, Nilsson S 1998 Differential response of estrogen receptor a and estrogen receptor β to partial estrogen agonists/antagonists. Mol Pharmacol 54:105-112; Couse JF, Lindsey J, Grandien K, Gustafsson J-A, Korach KS 1997 Tissue distribution and quantitative analysis of estrogen receptor-alpha and estrogen receptor-beta messenger ribonucleic acid in the wild type and ER-alpha knockout mouse. Endocrinology 138:4613-4621; Dotzlaw H, Leygue E, Watson PH, Murphy LC 1997 Expression of estrogen receptor-β in human breast tumors. J. Clin. Endocrinol. Metab. 82:2371-2377; Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson J 1998 Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 139:4252-4263; Kuiper GGJM, Gustafsson J-A 1997 The novel estrogen receptor-beta subtype: potential role in the cell- and promoter-specific actions of estrogens and anti-estrogens. FEBS Letters 410:87-90; Katzenellenbogen BS, Korach KS 1997 Editorial: A new actor in the estrogen receptor drama-Enter ER-β. Endocrinology 138:861-862; Montano MM, Jaiswal AK, Katzenellenbogen BS 1998 Transcriptional regulation of the human quinone reductase gene by antiestrogen-liganded estrogen receptor-α and estrogen receptor-β. J Biol Chem 273:25443-25449; Register TC, Shively CA, Lewis CE 1998 Expression of estrogen

receptor alpha and beta transcripts in female monkey hippocampus and hypothalamus. Brain Res 788:320-322; Register TC, Adams MR 1998 Coronary artery and cultured aortic smooth muscle cells express mRNA for both the classical estrogen receptor and the newly described estrogen receptor beta. J Steroid Biochem Molec Biol 64:187-191).

The estrogen subtypes, ERα and ERβ, are the products of two different genes. However, variant forms of both ER subtypes are known. ERβ variants having different N-terminal lengths that correspond to different transcriptional start sites are known (McInerney EM, Weiss KE, Sun J, Mosselman S, Katzenellenbogen BS 1998 Transcription activation by the human estrogen receptor subtypeβ (ERβ) studied with ERβ and ERα receptor chimeras. Endocrinology 139:4513-4522; Montano MM, Jaiswal AK, Katzenellenbogen BS 1998 Transcriptional regulation of the human quinone reductase gene by antiestrogen-liganded estrogen receptor-α and estrogen receptor-β. J Biol Chem 273:25443-25449). In addition, gene transcripts with deleted exons and alternate exon splicing, which may be translated into proteins, are known. These variant ER forms can have different transcription regulating activities, and can respond differently to different ER ligands (Chaidarun S, Alexander J 1998 A tumor-specific truncated estrogen receptor splice variant enhances estrogen-stimulated gene expression. Mol Endocrinol 12:1355-1366; Leygue ER, Watson PH, Murphy LC 1996 Estrogen receptor variants in normal human mammary tissue. J Natl Cancer Inst 88:284-290; Miksicek RJ, Lei Y, Wang Y 1993 Exon skipping gives rise to alternatively spliced forms of the estrogen receptor in breast tumor cells. Breast Cancer Res Treat 26:163-174; Pfeffer U, Fecarotta E, Vidali G 1995 Coexpression of multiple estrogen receptor variant messenger RNAs in normal and neoplastic breast tissue and in MCF-7 cells. Cancer Res 55:2158-2165; Zhang QX, Borg A, Fuqua SAW 1993 An exon 5 deletion variant of the estrogen receptor frequently co-expressed with wild-type estrogen receptor in human breast cancers. Cancer Res 53:5882-5884).

Various mutant forms of ERs have been characterized, and some of these show different responses to ER ligands (Wrenn CK, Katzenellenbogen BS 1993 Structure-function analysis of the hormone binding domain of the human estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast. J Biol Chem 268:24089-24098; Montano MM, Ekena KE, Krueger K, Keller AL, Katenellenbogen BS 1996 Human

estrogen receptor ligand activity inversion mutants: Receptors that interpret antiestrogens as estrogens and estrogens as antiestrogens and discriminate among different antiestrogens. Mol Endocrinol 10:230-242). ERs can be covalently modified by post-transcriptional events, such as phosphorylation, acetylation, and glycosylation. These modifications can also alter ER responsiveness to different ER ligands (Le Goff P, Montano MM, Schodin DJ, Katzenellenbogen BS 1994 Phosphorylation of the human estrogen receptor: Identification of hormone-regulated sites and examination of their influence on transcriptional activity. J Biol Chem 269:4458-4466; Kato SH, Endoh Y, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masucshige S, Gotoh Y, Nishida E, Kawashima H, Metzger D, Chambon P 1995 Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science 270:1491-1494).

Some of the actions of estrogens appear to be non-genomic, and may involve action through ERs in the cell membrane. Examples of such responses are stimulation of calcium flux regulation in bone cells and prolactin release from pituitary cells (Lieberherr M, Grosse B, Kachkache M, Balsan S 1993 Cell signaling and estrogens in female rat osteoblasts: A possible involvement of unconventional nonnuclear receptors. J Bone Miner Res 8:1365-1376; Marino M, Pallottini V, Trentalance A 1998 Estrogens cause rapid activation of IP3-PKC-a signal transduction pathway in HEPG2 cells. Biochem Biophys Res Commun 245:254-258; Mermelstein PG, Becker JB, Surmeier DJ 1996 Estradiol reduces calcium currents in rats neostriatal neurons via a membrane receptor. J Neuroscience 16:595-604; Pappas TC, Gametchu B, Watson CS 1995 Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. FASEB J 9:404-410; Pappas TC, Gametchu B, Yannariello-Brown J, Collins TJ, Watson CS 1994 Membrane estrogen receptors in GH3/B6 cells are associated with rapid estrogen-induced release of prolactin. Endocrine 2:813-822; Wehling M 1997 Specific, nongenomic actions of steroid hormones. Annu Rev Physiol 59:365-393; Zheng J, Ramirez VD 1997. Demonstration of membrane estrogen binding proteins in rat brain by ligand blotting using a 17b-estradiol-[^{125}I] bovine serum albumin conjugate. J Steroid Biochem Molec Biol 62:327-336). Although it is not known, as yet, which ER subtypes are important in regulating non-genomic responses, subtype-selective ER ligands may enable regulation of these responses in a tissue and cell selective manner.

Methods for selective regulation of cellular activities through ER employing the ER ligands of this invention can be used with variant, mutant and modified ERs as described herein and as known in the art. The interaction of ER ligands of this invention with variant, mutant and modified ERs can be assessed as described herein for ER.

The following examples are provided to further illustrate the invention and are in no way intended to limit the scope of the invention.

EXAMPLES

Example 1:

Measurement of relative ER Binding Affinities

A.

ER ligand binding assays can be performed as previously reported [Katzenellenbogen, J.A. et al. (1977) "Estrogen photoaffinity labels. 1. Chemical and radiochemical synthesis of hexestrol diazoketone and azide derivatives; photochemical studies in solution." Biochemistry **16**:1964-1970] using lamb and/or rat uterine cytosol diluted to approximately 1.5 nM of receptor, which was incubated with buffer of several concentrations of unlabeled competitor together with 10 nM [3H]estradiol for 18-24 hours. Free ligand was removed by adsorption to dextran-coated charcoal. Unlabeled competitors were prepared in 1:1 DMF:TEA to ensure solubility.

B.

Differential ligand binding affinities for ERα and ERβ can be determined by competitive radiometric binding assays using 10 nM [^{3}H]estradiol as tracer, and hydroxylapatite to adsorb bound receptor-ligand complex, as described previously [Carlson, K.E. et al. (1997) "Altered ligand binding properties and enhanced stability of a constitutively active estrogen receptor: evidence that an open-pocket conformation is required for ligand interaction," Biochemistry **36**:14897-14905]. Differential assays are performed using purified preparations of human ERα (amino acids 304-554) and ERβ (203-452) ligand binding domains expressed in *E. coli* or using full length ERα and ERβ expressed in *Baculovirus* (commerically available).

Example 2:

Cell assays for ER Ligand Activity

Compounds are tested as ER agonists/antagonists in transcriptional activation assays in cells expressing ERα or ERβ. Cells are transfected with an expression plasmid for ERα or ERβ together with an estrogen-responsive reporter gene construct e.g., $(ERE)_3$-pS2-CAT, and treated with increasing concentrations of the test compound or with estradiol for comparison. Reporter gene expression is a measure of the capacity of ER complexed with various compounds to activate transcription, and it is followed as a function of concentration of the test compound. Potency and agonist character in activating transcription is measured relative to activation of the same system by estradiol. The ability of the test compound to inhibit transcriptional activation by increasing concentrations of estradiol is also measured as a function of test compound concentration. The ability of a test compound to inhibit transcriptional activation by estradiol is a measure of antagonist character and antagonist potency of the test compound.

Transcriptional activation can be assessed with ERα or ERβ and in different cells types. Using the $(ERE)_3$-pS2-CAT reporter, CAT activity is measured as a function of the concentration of added test compound (typically ranging from 10^{-12} - 10^{-6} molar) in the presence or absence of the known stimulator (estradiol, typically ranging from 10^{-12} - 10^{-6} molar). Agonist and/or antagonist character can be selective for ERα and ERβ. Assays can be performed, for example, in human endometrial cancer (HEC-1) cells, Chinese hamster ovarian (CHO) cells, and HeLa cells. Agonist/antagonist character can also be assessed with various promoters, e.g., the estrogen-responsive pS2 promoter, the simple TATA promoter, a non-consensus lactoferrin estrogen-responsive promoter, a heterologous thymidine kinase promoter and the complement C3 promoter which is an estrogen-responsive promoter that contains a non-consensus ERE.

The agonist/antagonist character of a given test compound relative to a selected ER ligand, e.g., estradiol, can be assessed using the transcriptional activation assays described. A given compound may be a pure agonist activating expression and exhibiting no transcriptional inhibition, a pure antagonist suppressing stimulation of expression by known activators and not stimulating transcription themselves or a mixed agonist/antagonist

showing both types of behavior. Test compounds may exhibit selectivity in potency, where a given test compound stimulates transcription at lower concentration through one ER subtype than through the other ER subtype. Test compounds may exhibit selectivity in that they stimulate transcription or inhibit expression to a greater degree through one or the other of ERα and ERβ. Test compounds can exhibit a different level of potency for activation compared to inhibition of stimulation of gene expression.

Pyrazole compound **38b** was found to be an ERα potency selective agonist compared to estradiol when assayed in HEC-1 cells using $(ERE)_3$-pS2-CAT. It exhibited a 120-fold higher potency in activating transcription via ERα than via ERβ. In contrast, estradiol exhibits significantly lower activation selectivity between ERα and ERβ. Similar ERα potency-selective character was observed for this pyrazole in other cell types and with other estrogen-responsive promoters. Pyrazole compound **38b** was found to bind to ERα three-fold more strongly than to ERβ. Thus, differences in relative binding of the ligand does not fully account for the significantly higher (120-fold) selectivity for activation exhibited by the pyrazole with ERα compared that exhibited by the pyrazole with ERβ. These results suggest that factors beyond ligand-receptor interaction, such as receptor-coactivator interactions are likely important determinants of transcriptional potency.

CHEMICALS, MATERIALS, AND PLASMID CONSTRUCTIONS

Cell culture media were purchased from GIBCO (Grand Island, NY). Calf serum was from Hyclone Laboratories (Logan, UT) and fetal calf serum was from Atlanta Biologicals (Atlanta, GA). ^{14}C-Chloramphenicol (50-60 Ci/mmol) and [^{3}H]E_2 were from DuPont, NEN Research Products (Boston, MA). The expression vector for human ERα (pCMV5-hER) was constructed previously as described (Wrenn, C.D. and Katzenellenbogen, B.S. (1993), "Structure-function analysis of the hormone binding domain of the human estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast," J. Biol. Chem. **268**:24089-24098). The expression vector pCMV5-ERβ was constructed by inserting the full-length cDNA encoding human ERβ (530) residues, pNGV1-ERβ (Mosselmen et al. (1996) supra) and including the additional 53 N-terminal amino acids as found in Genebank accession number AF 051427), into the BamH1 site of pCMV5. The estrogen responsive reporter plasmids were $(ERE)_3$-pS2-CAT, constructed as described previously (Kraus, W.L.

et al. (1995), "Ligand-dependent, transcriptionally productive association of the amino-and carboxyl-terminal regions of a steroid hormone nuclear receptor," Proc. Natl. Acad. Sci. USA **92**:12314-12318), $(ERE)_2$-TATA-CAT [Wrenn, C.D. and Katzenellenbogen, B.S. (1993), "Structure-function analysis of the hormone binding domain of the human estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast," J. Biol. Chem. **268**:24089-24098], C3-Ti-LUC, which contains -1030 to +58 of the human complement C3 promoter fused to the firefly luciferase reporter gene (Norris, J.D. et al. (1996), "Identification of the sequences within the human complement 3 promoter required for estrogen responsiveness provides insight into the mechanism of tamoxifen mixed agonist activity," Mol. Endocrinol. **10**:1605-1616), and lactoferrin ERE-tk-CAT, which contains 2 copies of the non-consensus lactoferrin ERE fused to the thymidine kinase promoter and CAT reporter gene. The plasmid pCH110 (Pharmacia, Piscataway, NJ) or pCMVβ (Clontech, Palo Alto, CA) which contains the β-galactosidase gene, was used as an internal control for transfection efficiency. Expression vectors employed herein are commerically available or available through routine preparations using published information.

Cell culture and transient transfections

Human endometrial cancer (HEC-1) cells, chinese hamster ovary (CHO) cells and HeLa cells are maintained in culture and transfected as described (Wrenn, C.D. and Katzenellenbogen, B.S. (1993), "Structure-function analysis of the hormone binding domain of the human estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast," J. Biol. Chem. **268**:24089-24098; Montano, M.M. et al. (1995), "The carboxyl-terminal F domain of the human estrogen receptor: role in the transcriptional activity of the receptor and the effectiveness of antiestrogens as estrogen antagonists," Mol. Endocrinol. **9**:814-825; McInerney, E.M. and Katzenellenbogen, B.S. (1996), "Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation," J. Biol. Chem. **271**:24172-24178). Transfection of HEC-1 cells in 60-mm dishes utilizes 0.4 ml of a calcium phosphate precipitate containing 2.5 μg of the reporter gene plasmid, 100 ng of ER expression vector, and carrier DNA to a total of 5 μg DNA. CAT or luciferase activity, normalized for the internal control β-galactosidase activity, is assayed as described (Montano, M.M. et al. (1995), "The carboxyl-terminal F domain of the human estrogen receptor: role in the transcriptional activity of the receptor and

the effectiveness of antiestrogens as estrogen antagonists," Mol. Endocrinol. 9:814-825; McInerney, E.M. and Katzenellenbogen, B.S. (1996), "Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation," J. Biol. Chem. 271:24172-24178).

Example 3:

Chemical Syntheses

General Methods

All reactions using water- or air-sensitive reagents were conducted under an Ar atmosphere with dry solvents. Solvents were distilled under N_2 as follows: CH_2Cl_2 from CaH_2, THF from sodium benzophenone ketyl, DMF from $MgSO_4$, and Hexanes from $CaSO_4$. Triethyl amine was distilled over CaH_2. All other reagents were purchased from commercial suppliers and used without further purification. Reactions were all monitored by TLC, performed on 0.25 mm silica gel glass plates containing F-254 indicator. Visualization on TLC was achieved by UV light (254 nm), iodine vapors, or phosphomolybdic acid indicator. Flash chromatography was performed using Woelm 32-63 μm silica gel packing unless otherwise noted.

^1H NMR and ^{13}C NMR spectra were recorded on a Varian U400, Varian U500 or Varian INOVA 750. Electron ionization (EI) spectra were obtained using a Finnigan-MATCH5 spectrometer at 70 eV. Fast atom bombardment (FAB) were recorded on a VG ZAB-SE spectrometer. High pressure liquid chromatography (HPLC) was performed on a SpectraPhysics P100 solvent delivery system with ultraviolet detection at 254 nm. Elemental analysis was performed by the Microanalytical Service Laboratory at the University of Illinois.

Compound numbers listed refer to those in the Schemes.

General Demethylation Procedure using BBr_3. To a stirring solution of the methyl-protected heterocycle (1 eq.) in CH_2Cl_2 at -78 °C was added a solution of BBr_3 (4-5 eq.) as a 1N solution in CH_2Cl_2. The reaction were allowed to warm to room temperature and stirred for 18 h. After quenching with H_2O, the layers were separated and the aqueous layer

extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to afford the crude phenols. Flash chromatography afforded the demethylated products.

General Demethylation Procedure using BF_3·SMe_2. To a stirring solution of the methyl protected heterocycle (1 eq.) in CH_2Cl_2 (8 mL) at room temperature was added BF_3·SMe_2 complex (75 eq.). After stirring for 24 h, solvent and excess reagent were evaporated under nitrogen stream in hood. Residue was taken up in EtOAc and washed with H_2O and sat. NaCl. Organic extract was dried over Na_2SO_4, filtered and solvent removed under reduced pressure. The resulting residue was purified though a silica plug, eluting with EtOAc. Solvent evaporation afforded the deprotected products.

Imidazoles

4,5-Di(4-methoxyphenyl)-1H-imidazole **(2).** To 4,4'-dimethoxybenzil **(1)** (2.0g, 7.4 mmol) and *p*-formaldehyde (1.0 g, 11.1 mmol) was added formamide (50 mL). The bright yellow suspension was heated to reflux (220°C) for 2 h. The reaction mixture was then cooled to room temperature then to 0°C. The crystals that formed were filtered and recrystallized from EtOAc to afford **2** (2.4 g, 86%). mp 183-184°C (lit [16] mp 183-184°C);

4,5-Di(4-hydroxyphenyl)-1H-imidazole **(3).** Imidazole **2** (100 mg, 0.35 mmol) afforded **3** (52 mg, 59%) by the general BBr_3 demethylation procedure.

2,4,5-Tri(4-methoxyphenyl)-1H-imidazole **(4).** A suspension of 4,4'-dimethoxybenzil **(1)** (4.0 g, 15 mmol) and *p*-anisaldehyde (20 mL, 164 mmol) and formamide (100 mL) was heated to reflux (220°C) for 2 h, during which time the reaction mixture became homogeneous. The reaction was then cooled to 0°C and the precipitated product **4**, was filtered. The light yellow powder was recrystallized from MeOH/H_2O to afford 3.80 g of **4** [Hayes, J.F., Mitchell, M.B. & Wicks, C. (1994), "A novel synthesis of 2,4,5-triarylimidazoles," *Heterocycles* **38**, 575-585] (66%). mp 89-91°C (lit [Hayes, J.F., Mitchell, M.B. & Wicks, C. (1994), "A novel synthesis of 2,4,5-triarylimidazoles," *Heterocycles* **38**, 575-585] mp 88-94°C).

General N-Alkylation Procedure for Imidazoles. A solution of imidazole 4 (200 mg, 0.52 mmol) in THF (10 mL) and DMF (1.5 mL) was cooled to 5°C. NaH (31 mg, 0.78 mmol) was added as 60% dispersion in mineral oil. The reaction mixture was warmed to room temperature for 1 h and respective alkyl halide (0.04 mL, 0.62 mmol) was added. The resulting suspension was heated to reflux for 12 h, then cooled to room temperature. The light precipitate was filtered and the filtrate was concentrated under vacuum to a yellow solid which was flashed on silica (30% EtOAc/Hexanes) to afford alkylated products **5b-d** 1-Ethyl-2,4,5-tri(4-methoxyphenyl)-imidazole (**5b**), 1-Propyl-2,4,5-tri(4-methoxyphenyl)-imidazole (**5c**), 1-Butyl-2,4,5-tri(4-methoxyphenyl)-imidazole (**5d**) in 80-90% yields.

2,4,5-Tri(4-hydroxyphenyl)-1H-imidazole **(6a).** According to the general BBr_3 demethylation procedure above, imidazole 4 (3.0 g, 7.8 mmol) afforded **6a** as a green-orange solid that darkened upon exposure to air (1.8 g, 68%). mp 203-205 °C.

1-Ethyl-2,4,5-tri(4-hydroxyphenyl)-imidazole **(6b).** According to the general BBr_3 demethylation procedure above, imidazole **5b** (185 mg, 0.46 mmol) afforded **5b** (107 mg, 62%). mp 150-153 °C;

1-Propyl-2,4,5-tri(4-hydroxyphenyl)-imidazole **(6c).** According to the general BBr_3 demethylation procedure above, imidazole **5c** (170 mg, 0.40 mmol) afforded **6c** (86 mg, 55%). mp 172-175 °C.

1-Butyl-2,4,5-tri(4-hydroxyphenyl)-imidazole **(6d).** According to the general BBr_3 demethylation procedure above, imidazole **5d** (190 mg, 0.43 mmol) afforded **6d** (78 mg, 46 %). mp 153-155 °C (dec).

1-Ethyl-2,5-(4-methoxyphenyl)-4-phenyl imidazole **(11).** Azido-ketone **9** (50.0 mg, 0.187 mmol) and imine **10** (92.0 mg, 0.564 mmol) were dissolved in THF (15 mL). Et_3N (29.0 µL, 0.208 mmol) was added via syringe and reaction stirred at room temperature for 48 h. The reaction mixture was then poured into H_2O and extracted with CH_2Cl_2, organic fractions were pooled, dried over Na_2SO_4, filtered and solvent removed under reduced pressure. The intermediate, 2,5-dihydro-2-hydroxyimidazole, used in next step without

further purification or characterization, was taken up CH_2Cl_2 (10 mL). Solution was cooled to 0°C and TFA (14.4 μL, 0.187 mmol) was added via syringe. Reaction stirred at 0°C for 36 h. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with H_2O, sat. $NaHCO_3$, and sat. NaCl successively. Organic fraction was dried over Na_2SO_4, filtered and solvent removed under reduced pressure. Purification by flash column chromatography (1:2 EtOAc:Hexanes) and recrystallization from CH_2Cl_2/Hexanes afforded imidazole **11** as a white solid (24.6 mg, 34% yield from azide **9**).

1-Ethyl-2,5-(4-hydroxyphenyl)-4-phenyl imidazole **(12).** Imidazole **11** (12.0 mg, 0.031 mmol) was demethylated according to the general $BF_3 \cdot SMe_2$ procedure to afford imidazole **12** as an off-white powder (10.6 mg, 95%).

5-Ethyl-1,4-(4-methoxyphenyl)-2-phenyl imidazole **(16).** Keto-amide **15** (110.0 mg, 0.273 mmol) and ammonium acetate (105.0 mg, 1.362 mmol) were heated to reflux in acetic acid (10 mL) for 48 h. Acetic acid was removed under reduced pressure, resulting residue was taken up in EtOAc, washed with sat. $NaHCO_3$, H_2O, and sat. NaCl. Organic extracts were dried over Na_2SO_4, filtered and solvent removed. Product was purified by flash column chromatography (1:4 EtOAc:Hexanes) and recrystallization from CH_2Cl_2/Hexanes to give imidazole **16** as a white solid (25.7 mg, 25%).

5-Ethyl-1,4-(4-hydroxyphenyl)-2-phenyl imidazole **(17).** Imidazole **16** (25.0 mg, 0.065 mmol) was demethylated as outlined in general $BF_3 \cdot SMe_2$ procedure above to give deprotected imidazole **17** as an off-white powder (20.2 mg, 87%).

Thiazoles

2,4-Di(4-methoxyphenyl)-thiazole **(21a).** A suspension of thioamide **19** (1.3 g, 7.9 mmol) and α-bromo-4'-methoxy-acetophenone **(20)** (1.8 g, 7.9 mmol) in DMF (10 mL) was heated to reflux for 1h, until it became homogeneous. The heat was removed and the reaction was stirred for 15 h at room temperature. The reaction mixture was poured into H_2O (50 mL) and the solid precipitate was filtered to afford crude **21a**. Recrystallization from CH_3NO_2 afforded pure **21a** as light yellow crystals (1.8 g, 81%).

5-Ethyl-2,4-di(4-methoxyphenyl)-thiazole **(21b).** A suspension of thioamide **19** (975 mg, 5.8 mmol) and α-bromo-4'-methoxy-butyrophenone **(13)** (1.5 g, 5.8 mmol) in DMF (10 mL) was heated to reflux for 4 h, until it became homogeneous. The heat was removed and the reaction was poured into H_2O (50 mL). The water was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with sat. LiCl (10 mL), then brine (10 mL). After drying over $MgSO_4$, the reaction mixture was filtered, and concentrated to a yellow powder. Flash chromatography (10% EtOAc/Hexanes) afforded **21b** as a light yellow powder (1.1 g, 51%).

2,4-Di(4-hydroxyphenyl)-thiazole **(22a).** Thiazole **21a** (1.0 g, 3.6 mmol) was demethylated using BBr_3 as outlined in the general procedure above to afford **22a** (430 mg, 45%). mp 218-221 °C;

2,4-Di(4-hydroxyphenyl)-5-ethyl-thiazole **(22b).** Thiazole **21b** (1.0 g, 2.7 mmol) was demethylated according to the general BBr_3 procedure to afford **22b** (460 mg, 58%). mp 246-247 °C;

Oxazoles

2,4-(4-Methoxyphenyl)-5-phenyl oxazole **(28).** Azido-ketone **27** (0.18 g, 0.673 mmol) and *p*-anisaldehyde (0.25 mL, 2.05 mmol) were dissolved in THF (15 mL). Et_3N (94.0 µL, 0.674 mmol) was added via syringe and reaction stirred at room temperature for 48 h. The reaction mixture was then poured into H_2O and extracted with CH_2Cl_2, organic fraction was dried over Na_2SO_4, filtered and solvent removed under reduced pressure. Resulting intermediate 2,5-dihydro-5-hydroxyoxazole, used in next step without further purification or characterization, was taken up CH_2Cl_2 (10 mL). Solution was cooled to 0 °C and TFA (54.0 µL, 0.701 mmol) was added via syringe. Reaction stirred at 0 °C for 36 h. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with H_2O, sat. $NaHCO_3$, and sat. NaCl successively. Organic extracts were combined, dried over Na_2SO_4, filtered and solvent removed under reduced pressure . Purification by flash column chromatography (1:2 EtOAc:Hexanes) and recrystallization from CH_2Cl_2/Hexanes afforded oxazole **28** as a white solid (72.4 mg, 30% yield from azide **27**). mp 125-128 °C (lit. [Strzybny, P.P.E., van ES, T. & Backeberg, O.G.

(1969), "Reaction of α-acyloxyketones with ammonium acetate," *J. South African Chem. Inst.* **22**, 158-164] mp 126-127 °C);

2,4-(4-Hydroxyphenyl)-5-phenyl oxazole **(29).** Oxazole **28** (22.0 mg, 0.062 mmol) was demethylated according to the general $BF_3 \cdot SMe_2$ procedure above to give deprotected oxazole **29** as an off-white powder (18.8 mg, 93%).

2,5-(4-Methoxyphenyl)-4-phenyl oxazole **(30).** A solution of bromo-ketone **26** (87.0 mg, 0.285 mmol) and *p*-methoxybenzamide (43.0 mg, 0.285 mmol) in toluene was heated to reflux for 36 h. Toluene was removed under reduced pressure and resulting residue purified by flash column chromatography (1:4 EtOAc:Hexanes). Recrystallization of desired product from CH_2Cl_2/Hexanes afforded oxazole **30** as a colorless solid (52.9 mg, 52%). mp 147-149°C;

2,5-(4-Hydroxyphenyl)-4-phenyl oxazole **(31).** Oxazole **30** (22.0 mg, 0.062 mmol) was demethylated according to the general $BF_3 \cdot SMe_2$ procedure above to give deprotected oxazole **31** as an off-white powder (18.1 mg, 89%).

General Procedure for Pyrazole Synthesis. A suspension of diketone (1 eq.) and appropriate hydrazine hydrochloride (3-5 eq.) in a 3:1 mixture DMF:THF was heated to reflux for 16-24 h with reaction progress being monitored by TLC for disappearance of starting material. The reaction mixtures was cooled to room temperature and poured into iced sat. LiCl solution (10 mL) and EtOAc (10 mL). The layers were separated and the organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography (EtOAc/Hexanes systems) afforded the pyrazoles.

*3,5-Di(4-methoxyphenyl)-1H-pyrazol*e **(34a).** Diketone **33** (91 mg, 0.32 mmol) and hydrazine (0.1 mL, 3.2 mmol) were reacted as outlined in general pyrazole procedure to afford **34a** [van Steenis, J. (1946), "The nitration of dianisoylmethane and p-methoxydesoxybenzoin," *Chem. Ber.* , 29-46] as an off-white solid (32.6 mg, 38%). mp 172-175°C (lit [van Steenis, J. (1946), "The nitration of dianisoylmethane and p-methoxydesoxybenzoin," *Chem. Ber.* , 29-46] mp 174°C).

1-Phenyl-3,5-di(4-methoxyphenyl)-pyrazole **(34b).** Diketone **33** (100 mg, 0.35 mmol) and phenyl hydrazine hydrochloride (500 mg, 3.5 mmol) were reacted as outlined in general pyrazole procedure above to afford **34b** [Ando, W., Sato, R., Yamashita, M., Akasaka, T. & Miyazaki, H. (1983), "Quenching of singlet oxygen by 1,3,5-triaryl-2-pyraazolines," *J. Org. Chem.* **48**, 542-546] (30 mg, 25%). mp 159-161°C (lit [Ando, W., Sato, R., Yamashita, M., Akasaka, T. & Miyazaki, H. (1983), "Quenching of singlet oxygen by 1,3,5-triaryl-2-pyraazolines," *J. Org. Chem.* **48**, 542-546] mp 163°C).

1-Benzyl-3,5-di(4-methoxyphenyl)-pyrazole **(34c).** Diketone **33** (300 mg, 1.07 mmol) and benzylhydrazine dihydrochloride (335 mg, 2.13 mmol) were reacted as outlined in the general pyrazole procedure above to afford **34c** (179 mg, 45%).

1,3,5-Tri(4-methoxyphenyl)-pyrazole **(34d).** Diketone **33** (100 mg, 0.35 mmol) and 4-methoxyphenyl hydrazine hydrochloride (92.2 mg, 0.53 mmol) were reacted as outlined in the general pyrazole procedure above to afford **34d** as a white solid (112 mg, 85%).

3,5-Di(4-hydroxyphenyl)-1H-pyrazole **(35a).** Pyrazole **34a** (20 mg, 0.07 mmol) was demethylated with BBr_3 according to the general procedure to afford **35a** [Hergenrother, P.M. (1991), "New Developments in Thermally Stable Polymers," *Rec. Travl. Chim. Pays-Bas.* **110**, 481-491] as an off-white solid (11 mg, 63%).

1-Phenyl-3,5-di(4-hydroxyphenyl)-pyrazole **(35b).** Pyrazole **34b** (20 mg, 0.06 mmol) was demethylated with BBr_3 according to the general procedure to afford **34b** [Hergenrother, P.M. (1991), "New Developments in Thermally Stable Polymers," *Rec. Travl. Chim. Pays-Bas.* **110**, 481-491] as an off-white solid (11.5 mg, 58%).

1-Benzyl-3,5-di(4-hydroxyphenyl)-pyrazole **(35c).** Pyrazole **34c** (178 mg, 0.48 mmol) was demethylated with BBr_3 according to the general procedure to afford **35c** as a yellow film (100 mg, 66%).

1,3,5-Tri(4-hydroxyphenyl)-pyrazole **(35d).** Pyrazole **34d** (112 mg, 0.29 mmol) was demethylated with BBr_3 according to the general procedure to afford **35d** (44.8 mg, 45%) as an off-white solid.

4-Ethyl-3,5-di(4-methoxyphenyl)-1H-pyrazole **(37a).** Diketone **36** (100 mg, 0.32 mmol) and hydrazine (0.12 mL, 3.2 mmol) were reacted as outlined in the general pyrazole procedure above to afford **37a** as a while solid (69 mg, 70%).

1-Phenyl-4-ethyl-3,5-di(4-methoxyphenyl)-pyrazole (37b). Diketone **36** (100 mg, 0.35 mmol) and phenyl hydrazine hydrochloride (140 mg, 0.96 mmol) were reacted as outlined in the general pyrazole procedure above to afford **37b** as an orange solid (109 mg, 87%).

1-Benzyl-4-ethyl-3,5-di(4-methoxyphenyl)-pyrazole **(37c).** Diketone **36** (200 mg, 0.64 mmol) and benzylhydrazine dihydrochloride (188.2 mg, 0.97 mmol) were reacted as outlined in the general pyrazole procedure above to afford **37c** as colorless film (80 mg, 31%).

4-Ethyl-1,3,5-tri(4-methoxyphenyl)-pyrazole **(37d).** Diketone **36** (50 mg, 0.16 mmol) and 4-methoxyphenyl hydrazine hydrochloride (140 mg, 0.96 mmol) were reacted as outlined in the general pyrazole procedure above to afford **37d** as an orange solid (11 mg, 23%).

4-Ethyl-3,5-di(4-hydroxyphenyl)-1H-pyrazole **(38a).** Pyrazole **37a** (69 mg, 0.22 mmol) was demethylated according to the general BBr_3 procedure to afford **38a** as a white solid (35 mg, 57%).

1-Phenyl-4-ethyl-3,5-di(4-hydroxyphenyl)-pyrazole **(38b).** Pyrazole **37b** (100 mg, 0.26 mmol) was demethylated according to the general BBr_3 procedure to afford **38b** as a white solid (50 mg, 54%).

1-Benzyl-4-ethyl-3,5-di(4-hydroxyphenyl)-pyrazole **(38c).** Pyrazole **37c** (80 mg, 0.20 mmol) was demethylated according to the general BBr_3 procedure to afford **38c** contaminated with a brominated side product. The two compounds were separated using RPHPLC (30:70, H_2O:MeOH, Partisil ODS2 C-18 prep column) (50 mg, 54%).

4-Ethyl-1,3,5-tri(4-hydroxyphenyl)-pyrazole **(38d).** Pyrazole **37d** (10 mg, 0.03 mmol) was demethylated according to the general BBr_3 procedure to afford **38d** (9.9 mg, 100%).

3,5-Di(4-methoxyphenyl)isoxazole **(40).** To a solution of oxime **39** (1.0 g, 6 mmol) in THF (20 mL) at 0°C was added nBuLi (9.11 mL, 13.3 mmol) as a solution in Hexanes. The clear solution was stirred for 30 min at 0°C then methyl 4-methoxybenzoate (498 mg, 3 mmol) was added as a solution in THF (5 mL) over 5 min. The reaction mixture was stirred at 0°C for 30 min, then warmed to room temperature. 5 N HCl (10 mL) was added and the biphasic reaction mixture was brought to reflux overnight (12 h). Upon cooling to 0°C, isoxazole **40** [Ichinose, N., Mizuno, K., Tami, T. & Otsuji, Y. (1988), "A novel NO insertion into cyclopropane ring by use of $NOBF_4$. Formation of 2-isoxazolines," *Chem. Lett.* , 233-236] precipitated and was collected via filtration (450 mg, 27%). mp 174-177°C (lit [Ichinose, N., Mizuno, K., Tami, T. & Otsuji, Y. (1988), "A novel NO insertion into cyclopropane ring by use of $NOBF_4$. Formation of 2-isoxazolines," *Chem. Lett.* , 233-236] mp 176-177°C);

3,5-Di(4-hydroxyphenyl)isoxazole **(41).** Isoxazole **40** (300 g, 1.1 mmol) was demethylated according to the general BBr_3 procedure to afford **41** [Murthy, A.K., Rao, K.S.R.K.M. & Rao, N.V.S. (1968) "Isoxazolylphenols and their absorption spectra," *Aus. J. Chem.* **21**, 2315-2317] as a white solid (152 mg, 56 %). mp 267-269°C (lit [Murthy, A.K., Rao, K.S.R.K.M. & Rao, N.V.S. (1968) "Isoxazolylphenols and their absorption spectra," *Aus. J. Chem.* **21**, 2315-2317] mp 255°C);

General procedure for the synthesis of pyridazines:

A stirred solution of 1,4-dione (55.0 mg, 0.12 mmol) in hydrazine hydrate (5 mL) with a minimal amount of ethanol (to dissolve dione), was heated to reflux overnight. The reaction was allowed to cool to room temperature and then diluted with ethyl acetate (15 mL). The solution was transferred to a separatory and the aqueous layer washed with ethyl acetate. The organic extracts were pooled and washed with water, sat. sodium chloride, dried over sodium sulfate and filtered. Solvent was removed under reduced pressure to yield crude 4,5-dihydropyridazine. Flash column chromatography (1:4 EtOAc:hexanes) afforded pure 4,5-dihydropyridazine, which was taken up into methylene chloride and left exposed to air

overnight. Any remaining methylene chloride was removed under reduced pressure to afford crude pyridazine. Purification by flash column chromatography (1:1 EtOAc:hexanes) followed by recrystallization (EtOAc:Hex) gave pure pyridazine.

Methoxy-protected pyridazine were deprotected according to the BF_3SMe_2 general demethylation procedure as described above to afford pyridazine analogs.

General procedure for the synthesis of pyrimidines:

To a well-stirred solution of the ketone (1 mmol) and the nitrile (2.2 mmol) in an. dichloroethane (5 ml) was slowly added triflic anhydride (1.1 mmol). The reaction mixture was stirred at room temperature for 24hr, satd. bicarbonate solution was added and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with brine and dried (an. Na_2SO_4). The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography over silica gel using 30% ethyl acetate-hexane as eluent to furnish the pyrimidines.

General procedure for the synthesis of pyrazines:

Method A (Scheme 13E): A magnetically stirred solution of the α–diketone (1mmol) and the diamine (1 mmol) in acetic acid (1.5 ml) was refluxed for 3.5-4.0 hr. The reaction mixture was cooled, poured into ice and extracted with ethyl acetate (3x5ml). The combined organic phases were washed with brine and dried (an. Na_2SO_4). Evaporation of the solvent and purification of the residue over a silica gel column using 20% ethyl acetate-hexane as eluent furnished the pyrazines.

Method B (Scheme 13F) To a stirred mixture of the α–hydroxy ketones (1mmol each) in ethanol (6 ml) was added ammonium acetate (3 mmol). The reaction mixture was refluxed for 4hr, cooled and poured into ice, the precipitated solid filtered off and was washed with cold water. The residue was purified over a silica gel column using 20% ethyl acetate-hexane as eluent to furnish the mixture of pyrazines.

General protocol for deprotection of phenolic methyl ethers:

To a stirred solution of the protected pyrimidine (1 mmol) or pyrazine (1 mmol) in dichloromethane was added boron triflouride-dimethylsulfide complex (10 mmol/phenolic gp.) and the reaction stirred at room temperature for 24-36 hr. After quenching with water, the layers were separated and the aqueous layer extracted with ethyl acetate (3x10 ml). The combined extracts were washed with satd. bicarbonate solution, brine and dried (an. Na_2SO_4). Evaporation of the solvent and purification of the residue over a silica gel column using 50% ethyl acetate-hexane or ethyl acetate as eluent furnished the free phenolic pyrimidine or pyrazine.

General experimental procedure for synthesis of quinoxalines:

Step 1: A well stirred mixture of the *o*-phenylenediamine dihydrochloride (1 mmol) and the α-diketone (1 mmol) in acetic acid were refuxed for 3.5-4.0 hr. The reaction mixture was cooled, poured into ice and extracted with ethyl acetate (3x10 ml). The combined extracts were washed with brine, dried (an. Na_2SO_4) and concentrated. Purification of the residue over a silica gel column using 30% ethyl acetate-hexane as eluent furnished an ~1:1 unseparable mixture of the quinoxalines.

Step 2: To a magnetically stirred solution of the isomeric mixture of protected quinoxalines (1mmol) in dichloromethane was added boron triflouride-dimethyl sulfide (10 mmol/phenolic gp.) and the stirring continued for 2 days at room temperature. After quenching with water, the layers were separated and the aqueous layer extracted with ethyl acetate (3x10 ml). The combined extracts were washed with satd. bicarbonate solution, brine and dried (an. Na_2SO_4). Evaporation of the solvent and purification of the residue over a silica gel column using ethyl acetate as eluent furnished the deprotected quinoxalines.

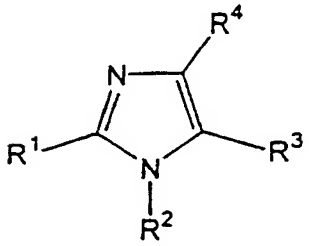
Those of ordinary skill in the art will appreciate that starting materials, reagents, reaction conditions, methods, techniques, purification and isolation methods other those specifically detailed herein can be employed or readily adapted in view of well-know principles to make and use the compounds of this invention. All art-known equivalents of

starting materials, reagents, reaction conditions, methods, techniques, purification and isolation methods described herein are intended to be encompassed by this invention.

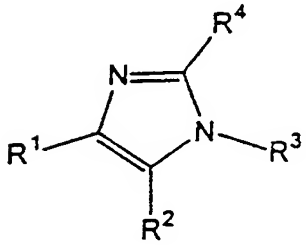
All references cited herein are incorporated in their entirety herein to the extent that they are not inconsistent with the disclosure herein

TABLE 1: EXEMPLARY STRUCTURES OF FIVE-MEMBERED RING CORES

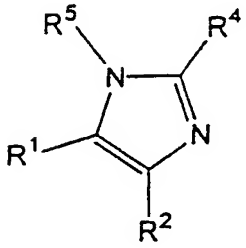
IMIDAZOLES



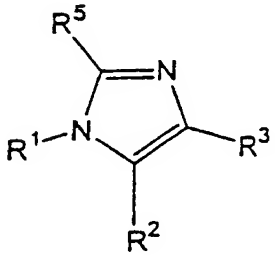
IM1



IM2

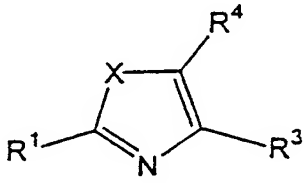


IM3

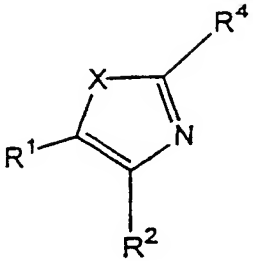


IM4

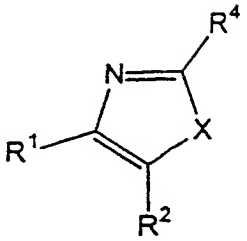
OXAZOLES/THIAZOLES



X = O, OA1
X = S, TA1



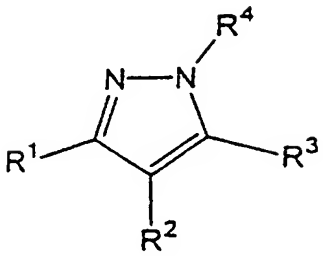
X = O, OA2
X = S, TA2



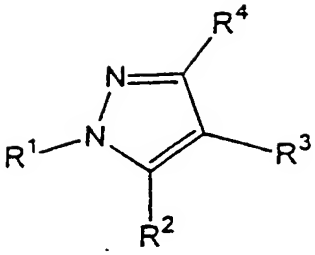
X = O, OA3
X = S, TA3

Table 1 (continued)

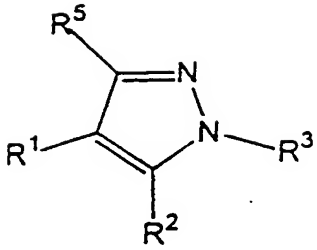
PYRAZOLES



PA1

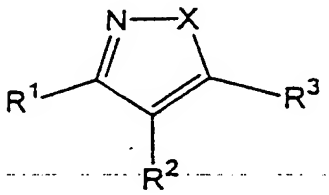


PA2

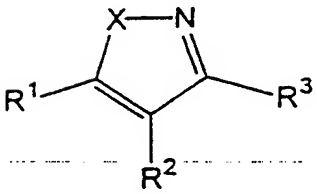


PA3

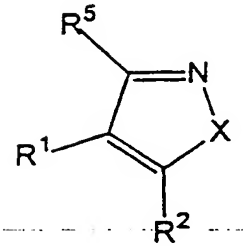
ISOXAZOLE/ISOTHIAZOLE



X = O, IO1
X = S, IS1

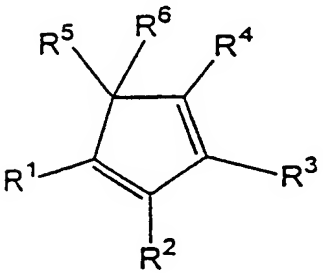


X = O, IO2
X = S, IS2

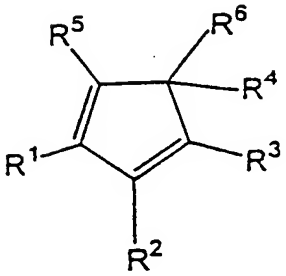


X = O, IO3
X = S, IS3

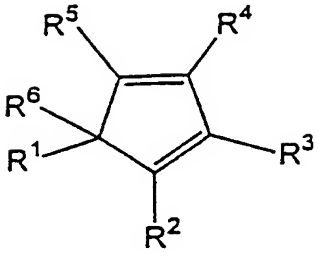
CYCLOPENTADIENES



C1



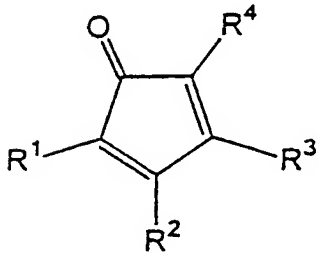
C2



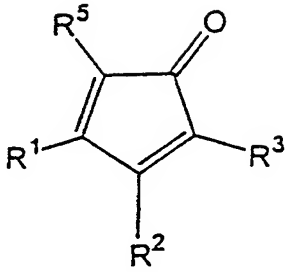
C3

Table 1(continued)

CYCLOPENTADIENEONES



CD1



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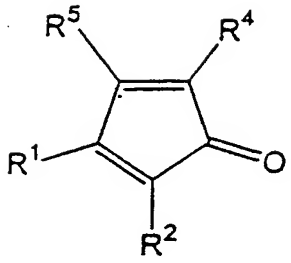
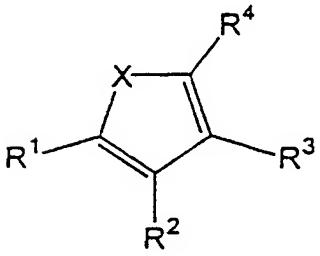
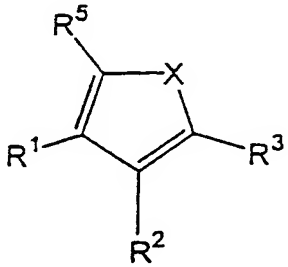


CD3

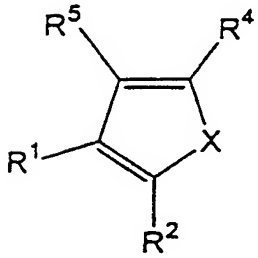
FURANS/THIOPHENES



X = O, F1
X = S, T1

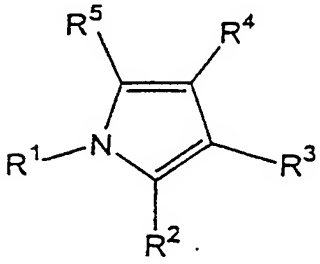


X = O, F2
X = S, T2

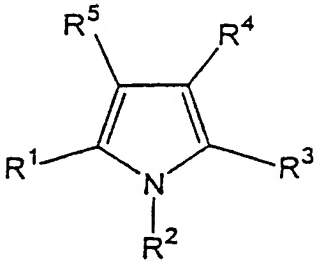


X = O, F3
X = S, T3

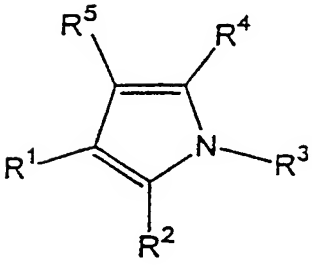
PYRROLES



PR1



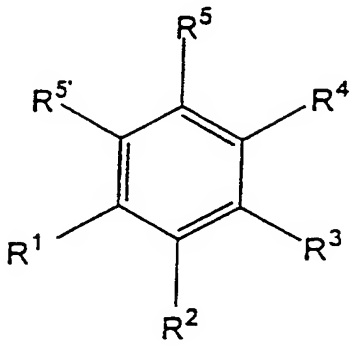
PR2



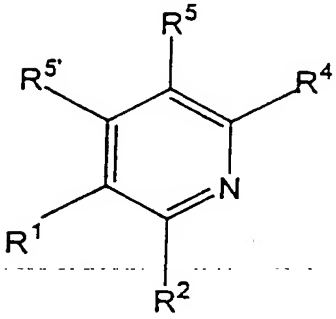
PR3

TABLE 2: EXEMPLARY STRUCTURES OF SIX-MEMBERED RING CORES

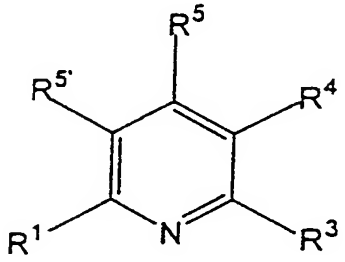
BENZENES



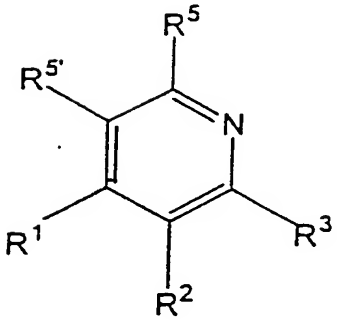
PYRIDINES



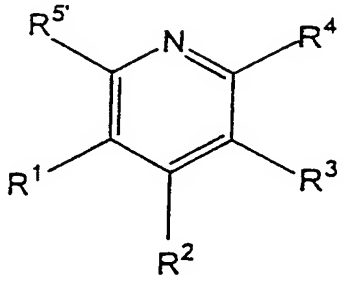
PY1



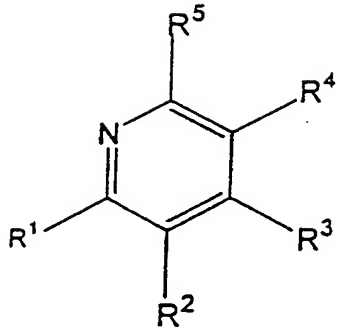
PY2



PY3



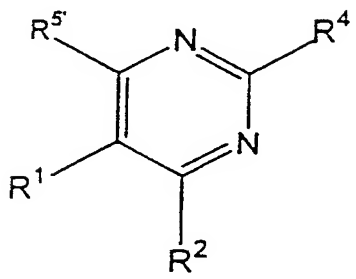
PY4



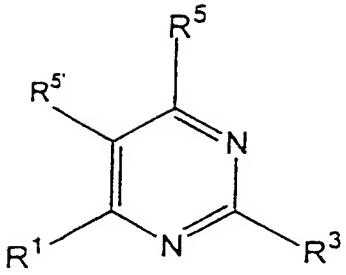
PY5

Table 2 (Continued)

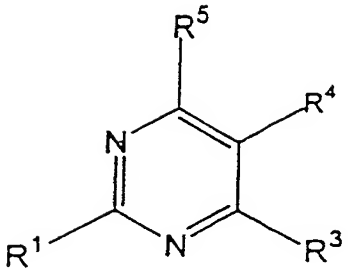
PYRIMIDINES



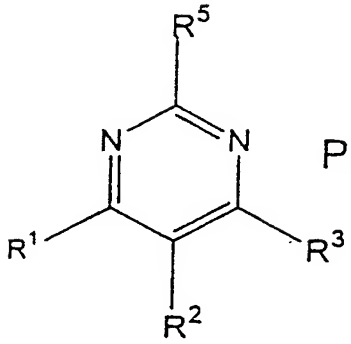
PM1



PM2

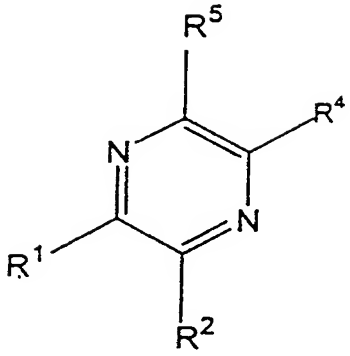


PM3

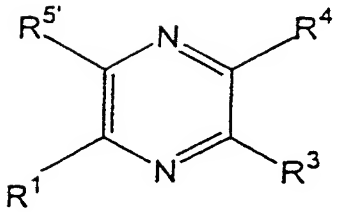


PM4

PYRAZINES



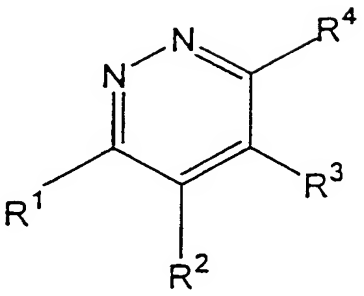
PZ1



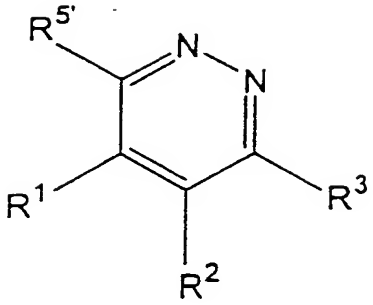
PZ2

Table 2(Continued)

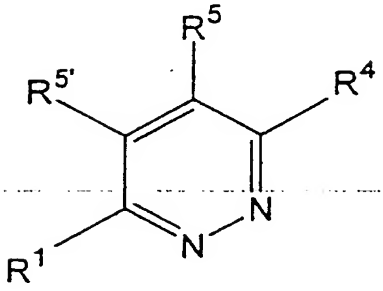
PYRIDAZINES



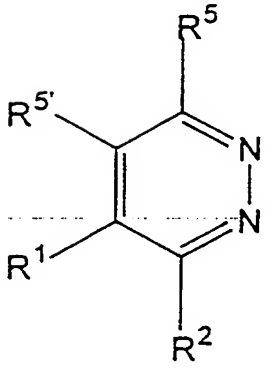
PZD1



PZD2



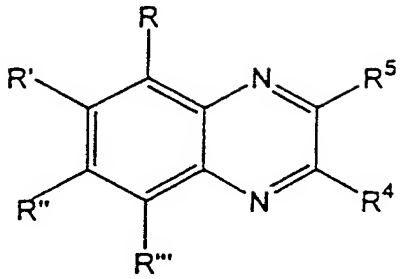
PZD3



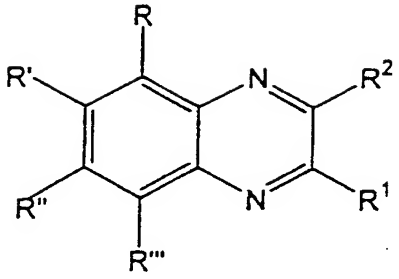
PZD4

Table 2 (Continued)

Quinoxalines

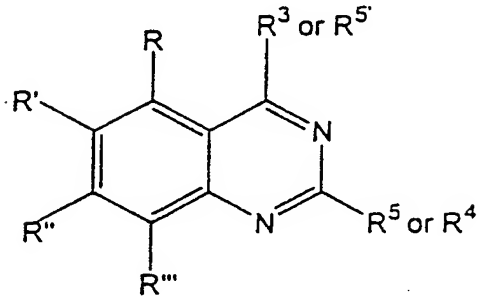


QX1

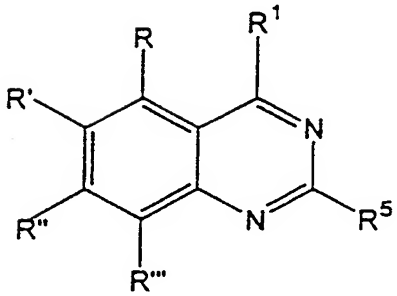


QX2

Quinazolines



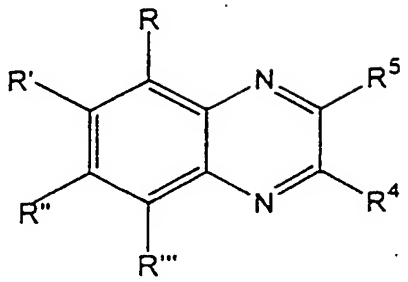
QZ1



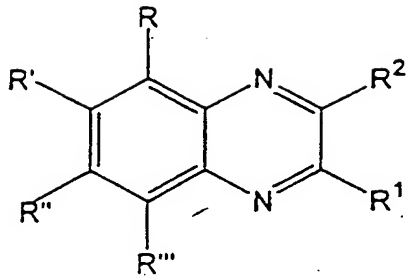
QZ2

Table 2(Continued)

Cinnolines

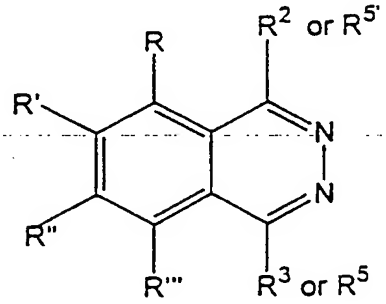


CN1

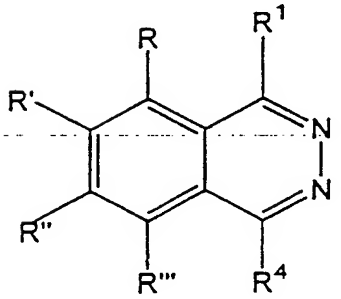


CN2

Phthalazines

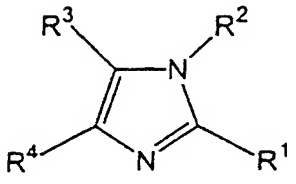


PH1



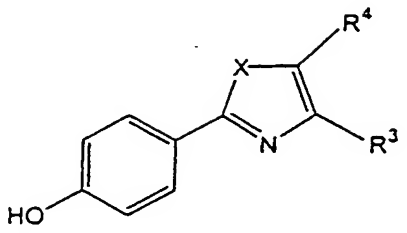
PH2

Table 3: ESTROGEN RECEPTOR BINDING DATA FOR IMIDAZOLES 3, 6A-D, 12 AND 17



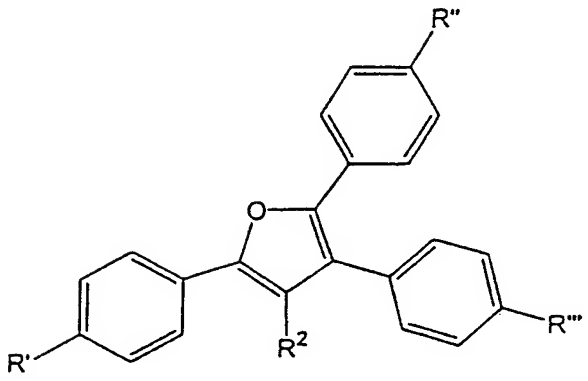
Compound	R^4	R^3	R^2	R^1	RBA
3	4'-HO-C_6H_4	4'-HO-C_6H_4	H	H	<0.001
6a	4'-HO-C_6H_4	4'-HO-C_6H_4	H	4'-HO-C_6H_4	0.007
6b	4'-HO-C_6H_4	4'-HO-C_6H_4	C_2H_5	4'-HO-C_6H_4	0.38
6c	4'-HO-C_6H_4	4'-HO-C_6H_4	C_3H_7	4'-HO-C_6H_4	0.62
6d	4'-HO-C_6H_4	4'-HO-C_6H_4	C_4H_9	4'-HO-C_6H_4	0.17
12	C_6H_5	4'-HO-C_6H_4	C_2H_5	4'-HO-C_6H_4	0.25
17	4'-HO-C_6H_4	C_2H_5	4'-HO-C_6H_4	C_6H_5	0.37

Table 4: ESTROGEN RECEPTOR BINDING DATA FOR THIAZOLES 22AB AND OXAZOLES 29 AND 31



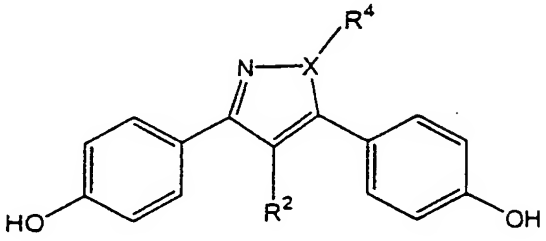
Compound	X	R^3	R^4	RBA
22a	S	4'-HO-C_6H_4	H	0.018
22b	S	4'-HO-C_6H_4	C_2H_5	0.041
29	O	4'-HO-C_6H_4	C_6H_5	<0.001
31	O	C_6H_5	4'-HO-C_6H_4	0.027

Table 5: ER BINDING AFFINITIES FOR EXEMPLARY FURANS



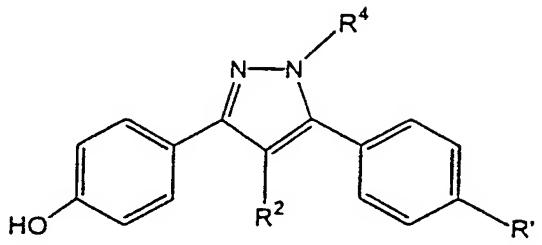
	R^2	R′	R″	R‴	BA(%)	ERα(%)	ERβ(%)
201	C_2H_5	OH	H	OH	5.01	67.6	6.31
202	n-C_3H_7	OH	H	OH	3.89		
203	C_2H_5	OH	OH	OH	5.89	214	3.02
204	n-C_3H_7	OH	OH	OH	9.33	85.1	2.4
	H	OH	H	OH	0.04		
	H	OH	OH	H	0.13		
200	C_2H_5	OH	OH	H	0.71	15.1	2.51

Table 6A: ESTROGEN RECEPTOR BINDING AFFINITY DATA FOR PYRAZOLES AND ISOXAZOLE



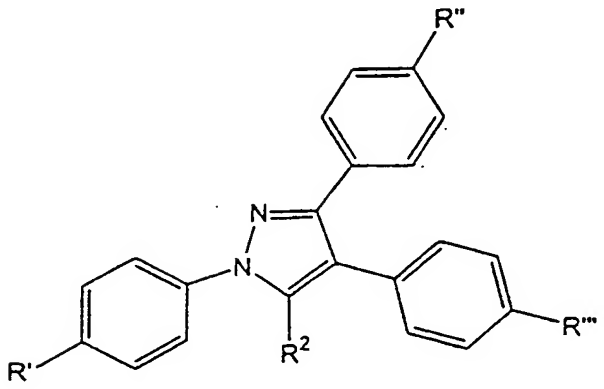
Compound	X	R^4	R^2	RBA
35a	N	H	H	0.009
35b	N	C_6H_5-	H	0.028
35c	N	$C_6H_5CH_2$-	H	<0.007
35d	N	$pHOC_6H_4$-	H	0.059
38a	N	H	C_2H_5	0.015
38b	N	C_6H_5-	C_2H_5	14.0
38c	N	$C_6H_5CH_2$-	C_2H_5	0.150
38d	N	$pHOC_6H_4$-	C_2H_5	19.0
41	O	--	C_2H_5	0.006

Table 6B: ESTROGEN RECEPTOR BINDING AFFINITIES REPRESENTATIVE FOR PYRAZOLES



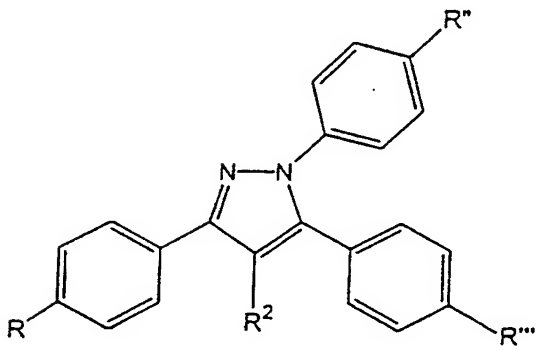
R_2	R'	R_4	%RBA
H	OH	H	0.009
H	OH	C_6H_5	0.028
H	OH	$C_6H_5CH_2$	<0.007
H	OH	p-HOC_6H_4	0.059
C_2H_5	OH	H	0.015
C_2H_5	OH	C_6H_5	14
C_2H_5	OH	$C_6H_5CH_2$	0.47
C_2H_5	OH	p-HOC_6H_4	20
CH_3	OH	C_6H_5	1.6
C_2H_5	OH	CH_2CH_2OH	1.2
C_3H_7	OH	C_6H_5	25

Table 7: ER BINDING AFFINITIES FOR EXEMPLARY PYRAZOLE ISOMERS

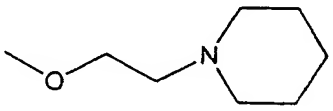


	R′	R″	R‴	R^2	RBA(%)
210	H	H	OH	C_2H_5	0.008 ± 0.005
211	OH	H	H	C_2H_5	0.43 ± 0.07
212	OH	H	OH	C_2H_5	5.6
213	OH	H	OH	n-C_3H_7	15
214	OH	OH	OH	C_2H_5	13

Table 8: ER BINDING AFFINITIES FOR PYRAZOLES WITH BASIC SIDE GROUPS.

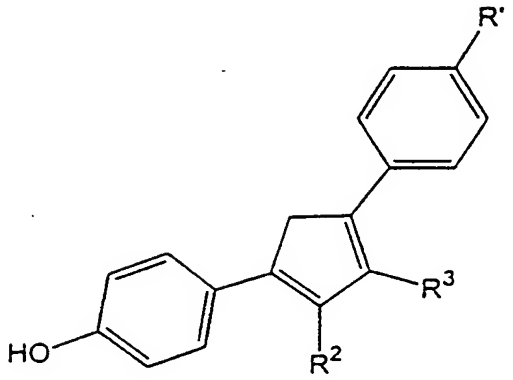


PI =



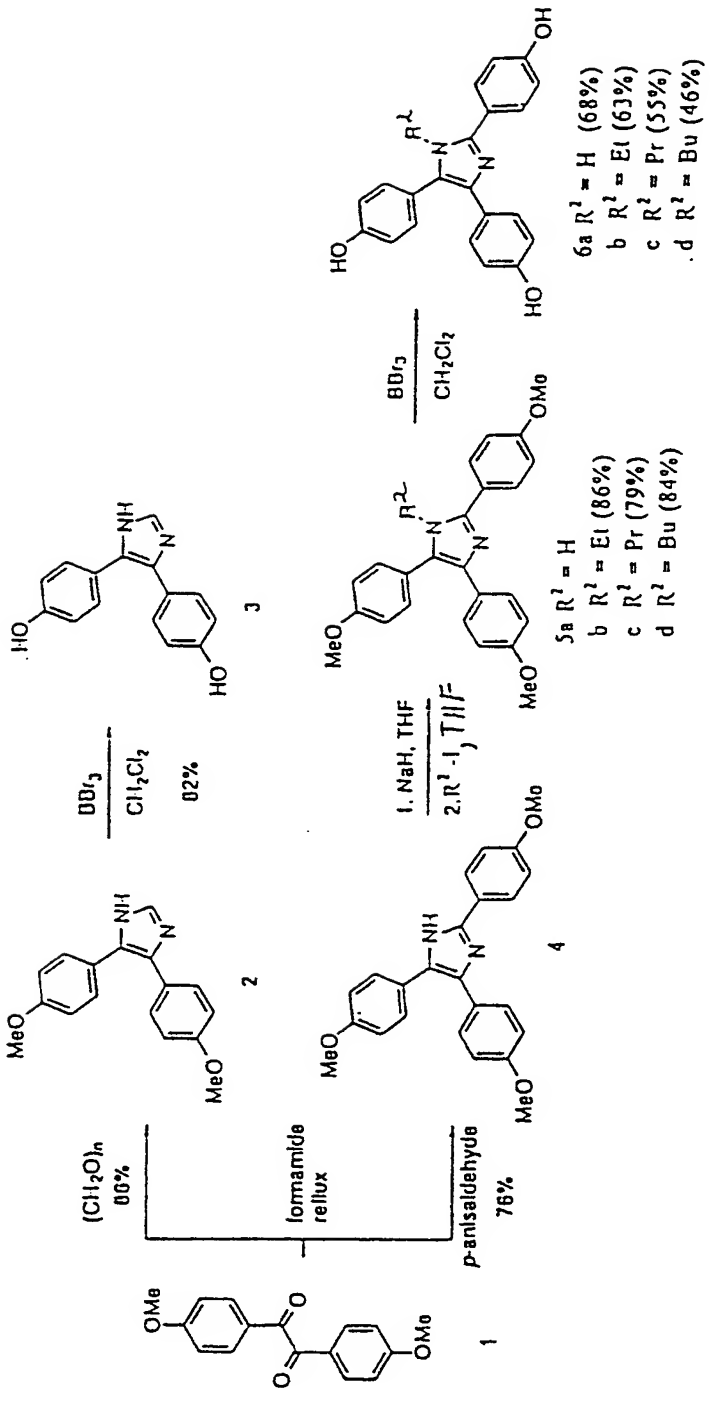
	R	R"	R'''	R^2	RBA% cytosol	$RBA_{ER\alpha}$	$RBA_{ER\beta}$
	OH	PI	OH	C_2H_5	2.0		
	OH	H	OH	PI	0.013		
	PI	OH	OH	C_2H_5	0.40		
301	OH	OH	PI	C_2H_5	32 ± 6.4	5.1 ± 1.6	0.18 ± 0.17

Table 9: RELATIVE ER BINDING AFFINITY DATA FOR CYCLOPENTADIENES



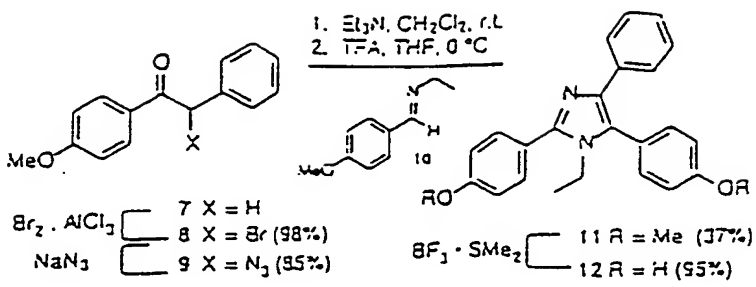
	R'	R^2	R^3	RBA (%)	RBA ERα	RBA ERβ
	H	C_2H_5	C_2H_5	0.028	0.372	0.355
	OH	C_2H_5	C_2H_5	1.047		
	H	C_2H_5	C_6H_5	0.05	1.26	0.50
	OH	C_2H_5	C_6H_5	1.20	8.32	7.08
235	OH	C_2H_5	p-OH-C_6H_5	8.91	5.25	1.66
	OH	n-C_3H_7	C_6H_5	0.06	0.33	0.708
	OH	n-C_3H_7	p-OH-C_6H_5	1.12	8.51	1.66

Scheme 1A



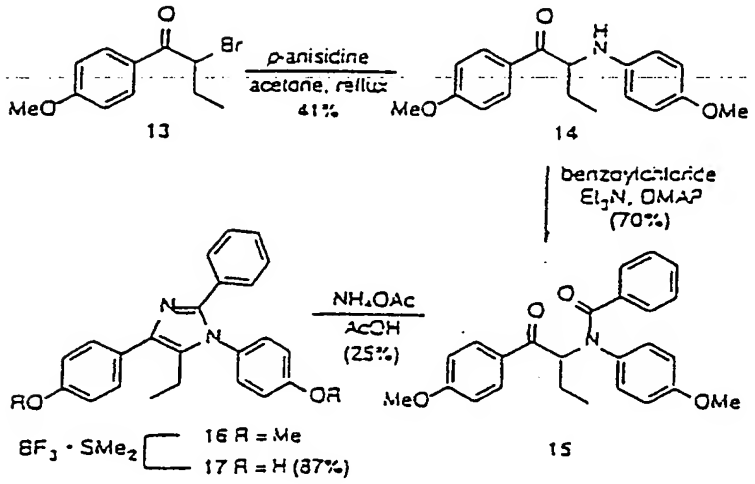
where $R^1 = R^3 = R^4 = p\text{-}OH\text{-}C_6H_4$- or p-MeO-$C_6H_4$-.

Scheme 1B



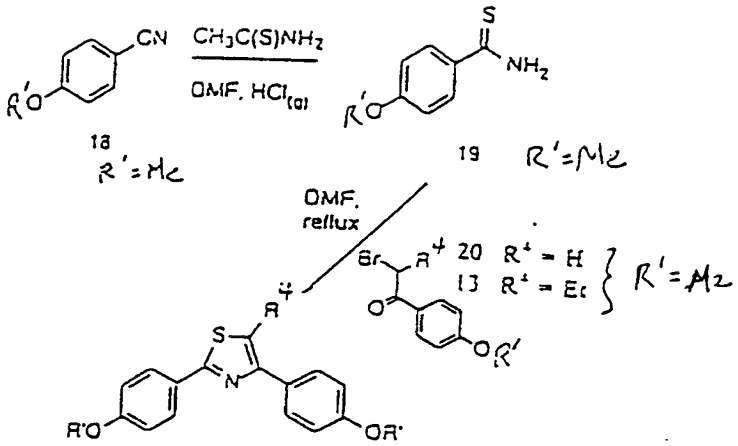
IM1 where $R^1 = R^3 =$ p-OH-C_6H_4 or p-MeO-C_6H_4
$R^4 = C_5H_5$
$R^2 =$ Et

Scheme 2



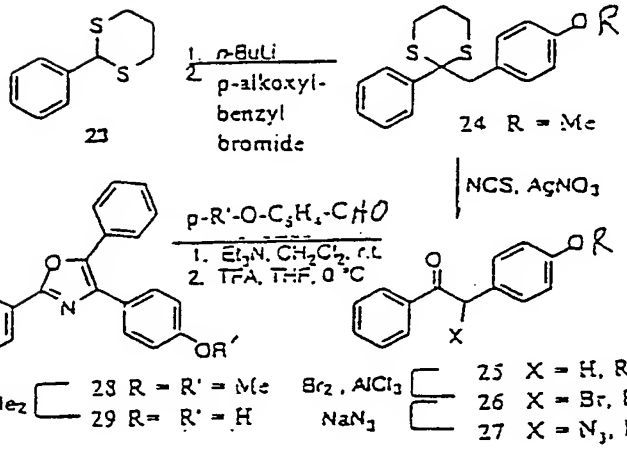
IM2 where $R^1 = R^3 =$ p-OH-C_6H_4 or p-MeO-C_6H_4
$R^4 = C_5H_5$-
$R^2 =$ Et

Scheme 3



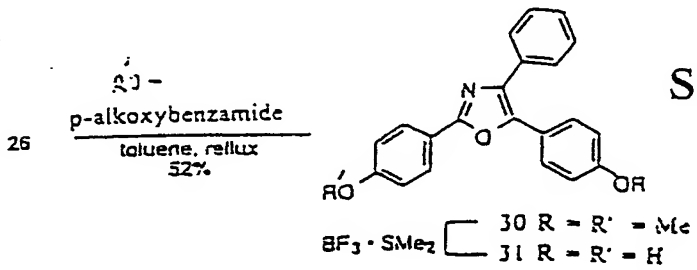
TA1 where $R^1 = R^3 =$ p-OH-C_6H_4 or p-MeO-C_6H_4
$R^4 =$ H or Et

Scheme 4A

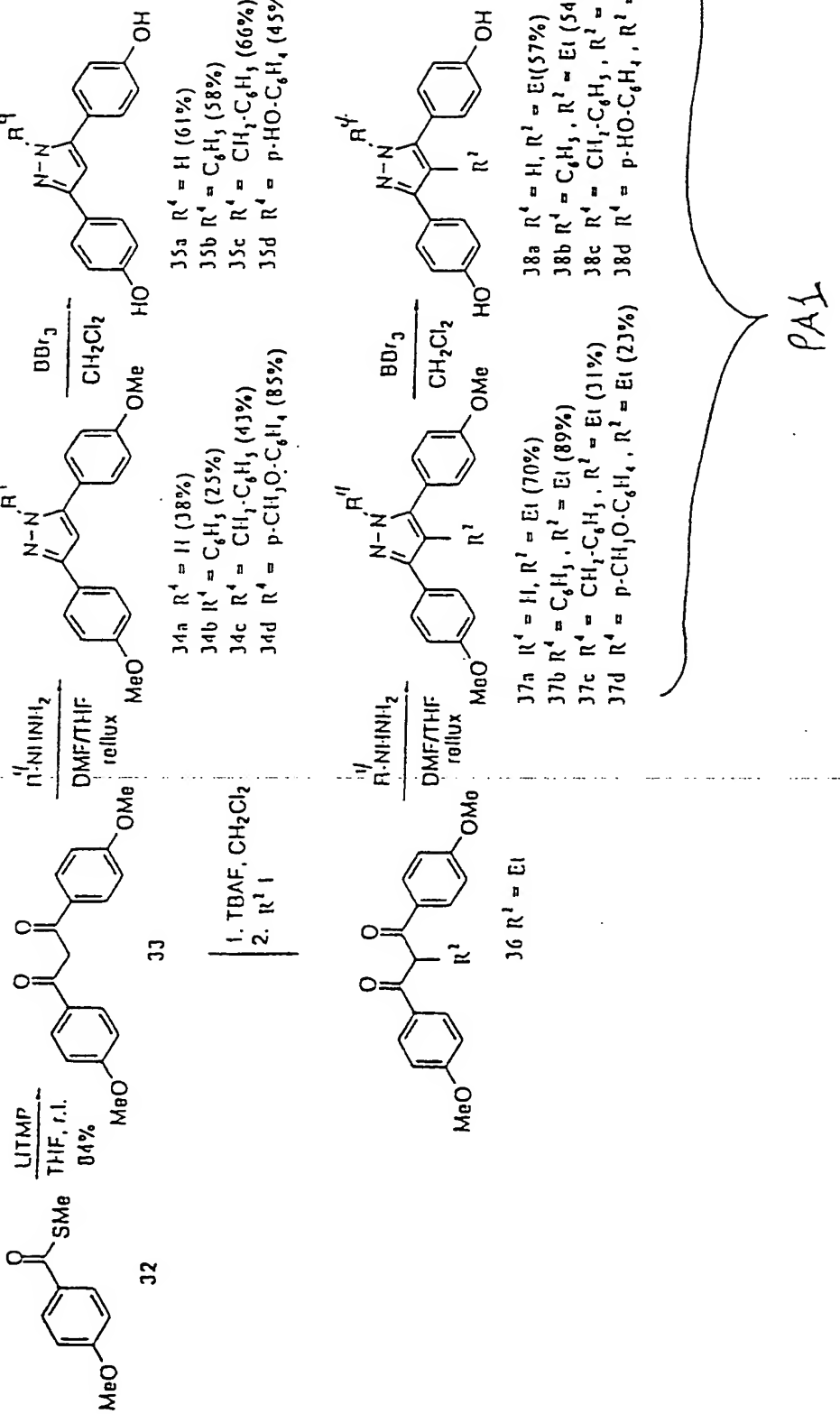


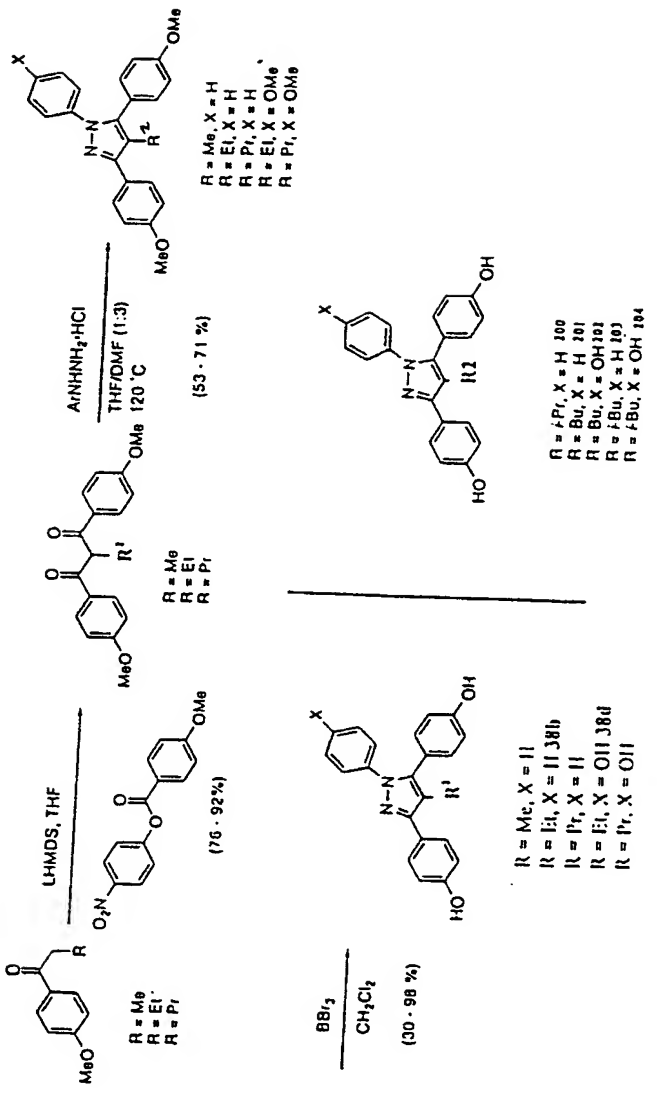
OAI R^1 = p-R-O-C_6H_4
R^3 = p-R'-O-C_6H_4
$R^4 = C_6H_5$

Scheme 4B



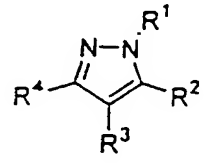
Scheme 5A



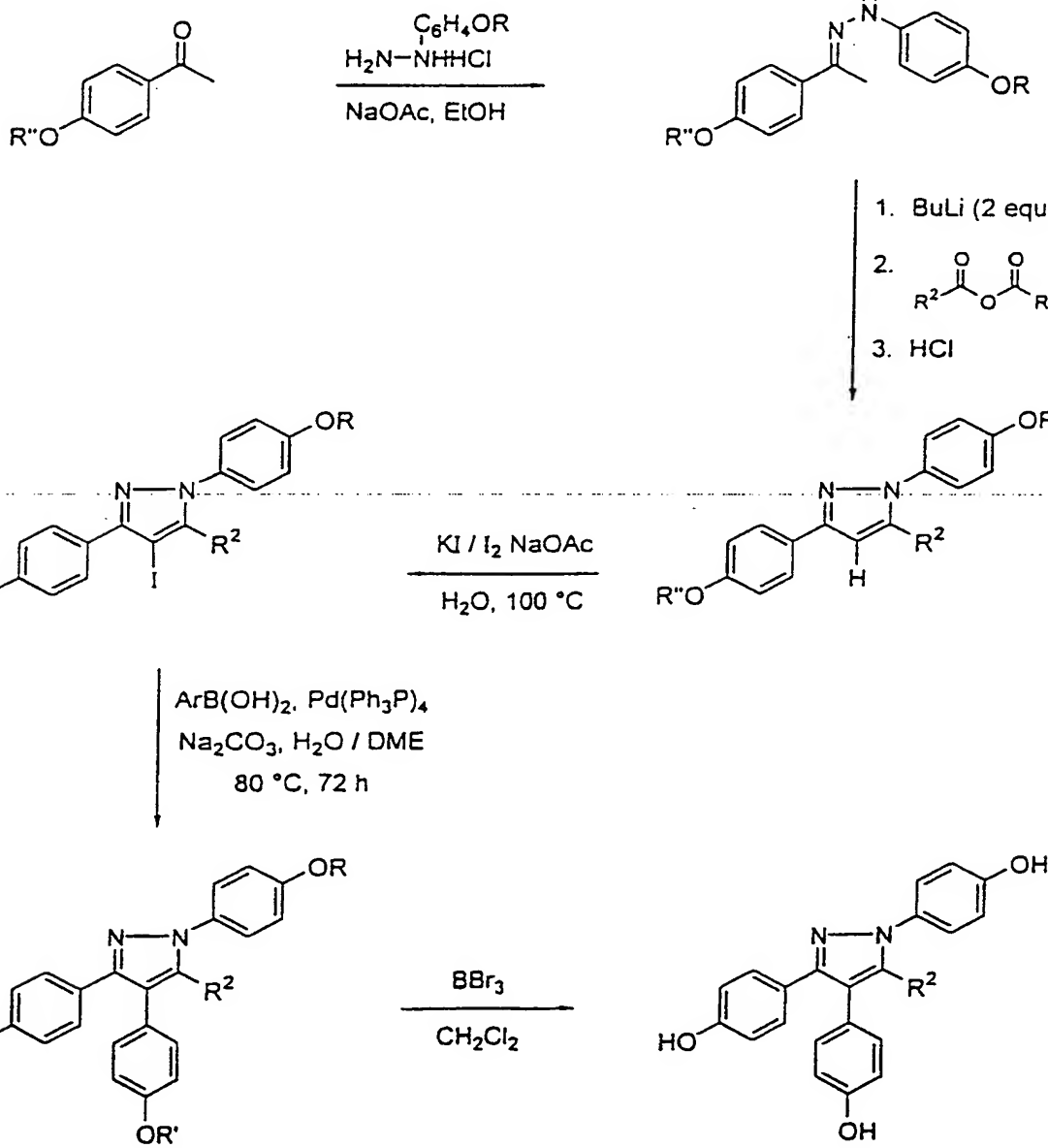


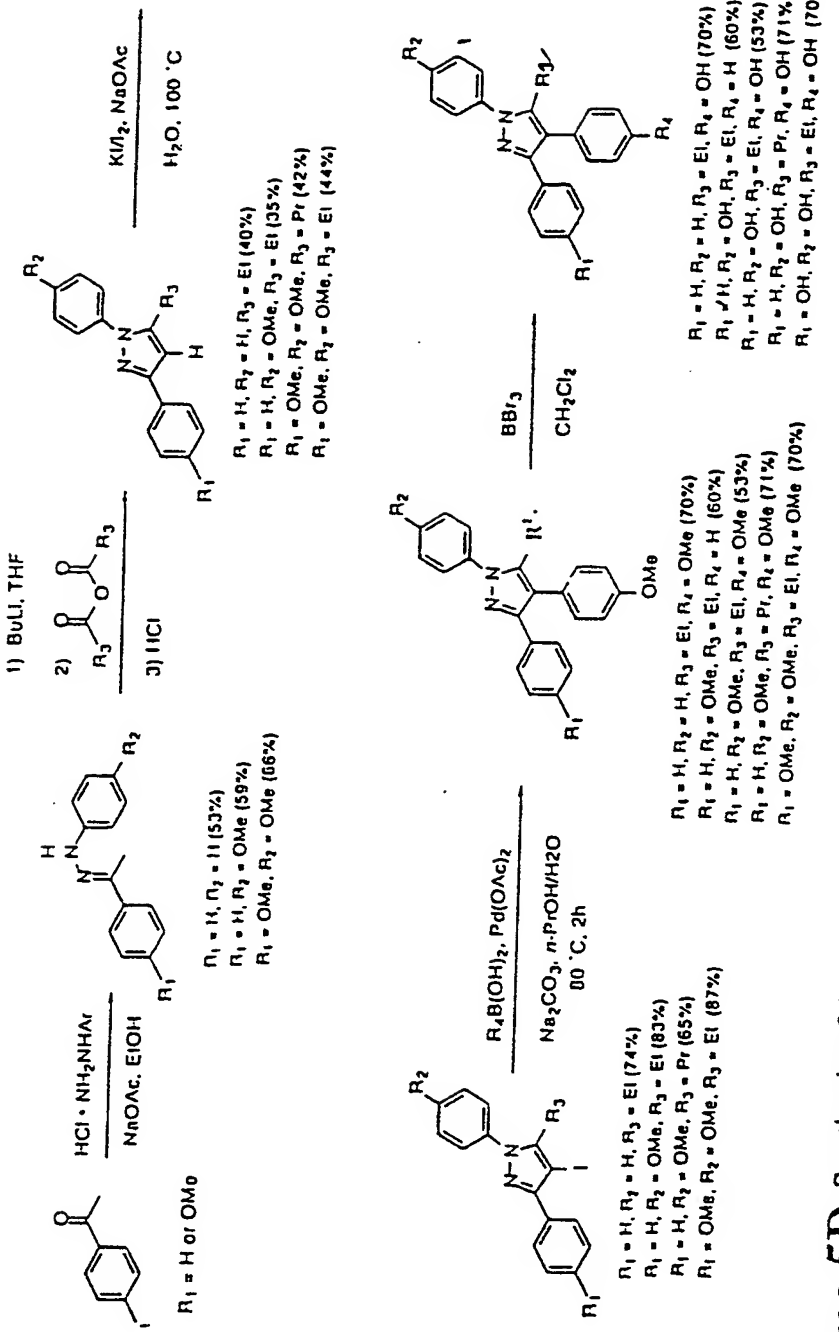
Scheme 5B Synthesis of C(4) alkyl pyrazole analogs.

Scheme 5C

For synthesis of: PA2 

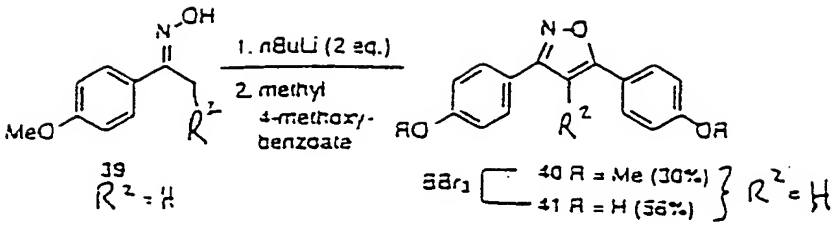
particularly: $R^1 = C_6H_4\text{-}OR$, $R = CH_3$, H
$R^3 = X$, $C_6H_4\text{-}OR'$, $R' = CH_3$, H
$R^4 = C_6H_5$, $C_6H_4\text{-}OR''$, $R'' = CH_3$, H



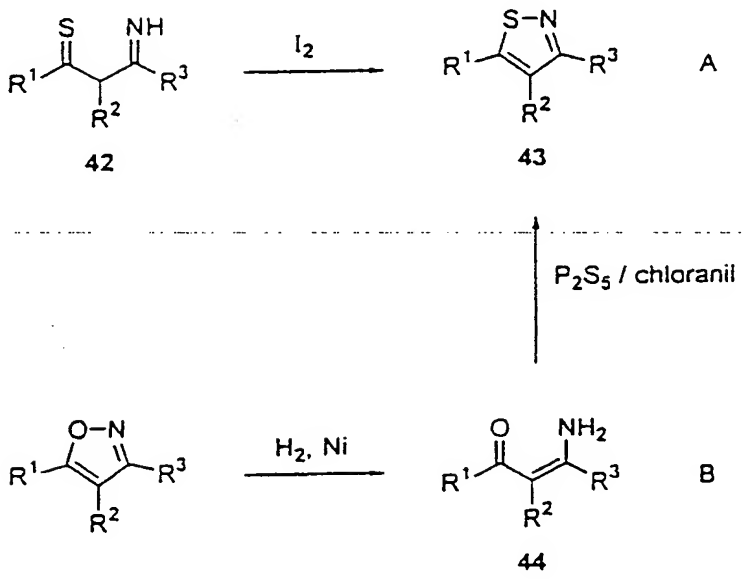


Scheme 5D Synthesis of 1,3,4-triaryl-5-alkylpyrazoles.

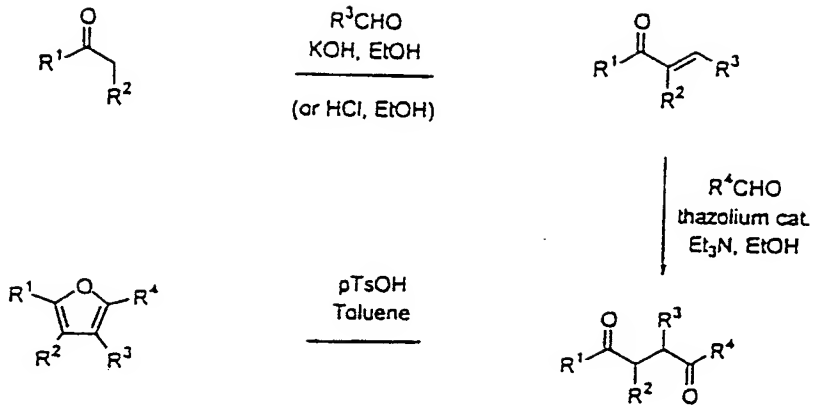
Scheme 6



Scheme 7

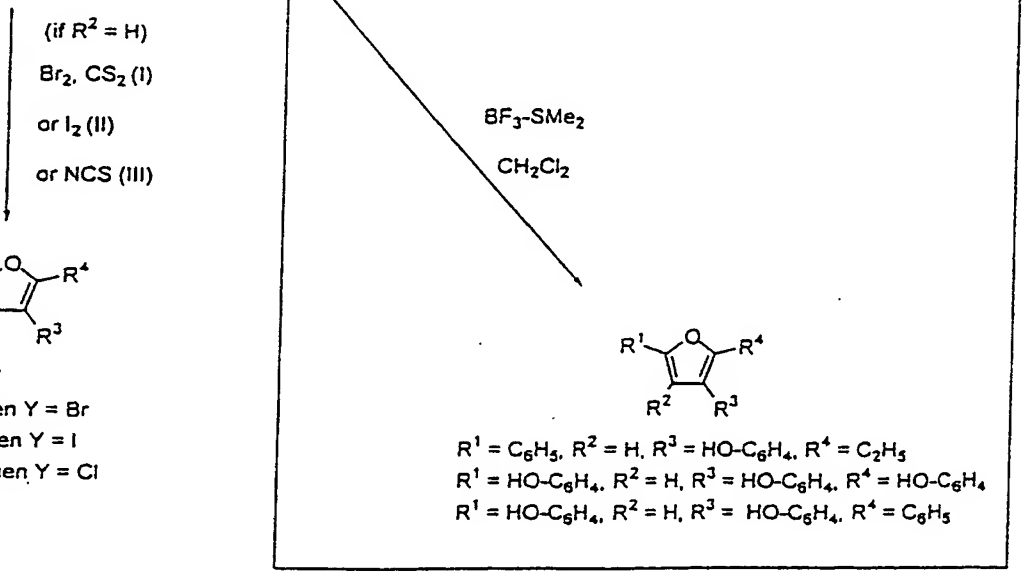


Scheme 8



57 $R^1 = C_6H_5$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = C_2H_5$
58 $R^1 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = p\text{-}CH_3O\text{-}C_6H_4$
59 $R^1 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = C_6H_5$

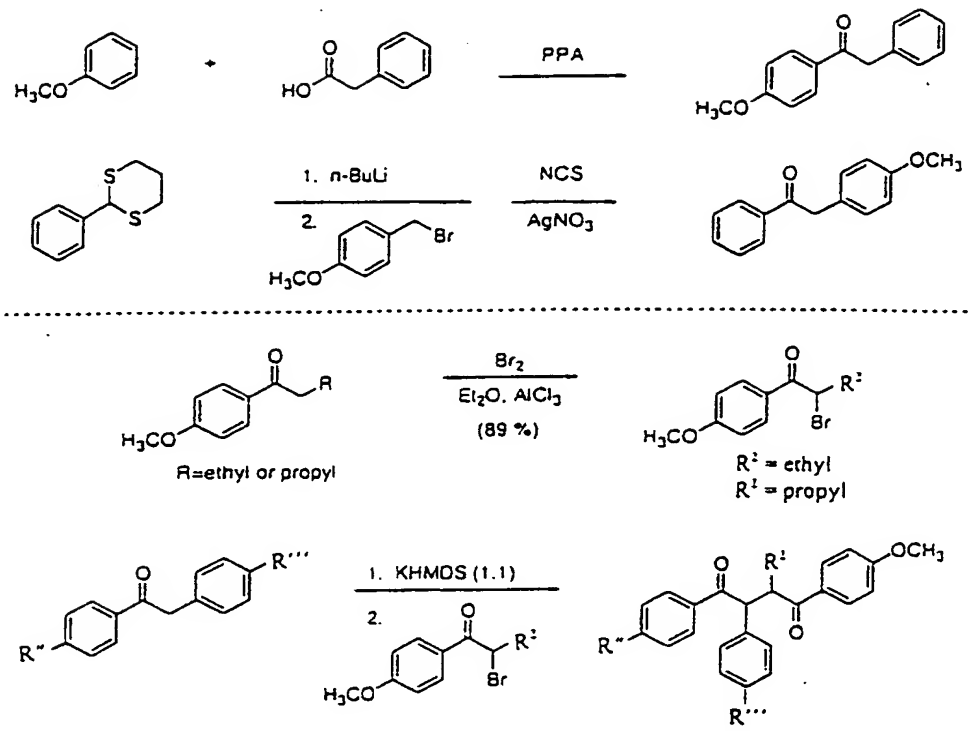
53 $R^1 = C_6H_5$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = C_2H_5$
54 $R^1 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = p\text{-}CH_3O\text{-}C_6H_4$
56 $R^1 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^4 = C_6H_5$



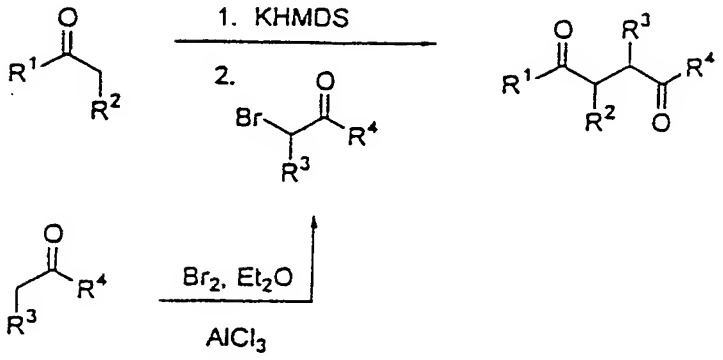
$R^2 = Y$
if I, then Y = Br
if II, then Y = I
if III, then Y = Cl

$R^1 = C_6H_5$, $R^2 = H$, $R^3 = HO\text{-}C_6H_4$, $R^4 = C_2H_5$
$R^1 = HO\text{-}C_6H_4$, $R^2 = H$, $R^3 = HO\text{-}C_6H_4$, $R^4 = HO\text{-}C_6H_4$
$R^1 = HO\text{-}C_6H_4$, $R^2 = H$, $R^3 = HO\text{-}C_6H_4$, $R^4 = C_6H_5$

Scheme 8A

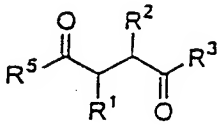


Scheme 8B alternative 1,4-dione synthesis, particularly: $R^1 = C_6H_5$, C_6H_4-OR, R = CH_3, H
$R^2 = C_6H_5$, C_6H_4-OR', R' = CH_3, H
$R^4 = C_6H_5$, C_6H_4-OR", R" = CH_3, H



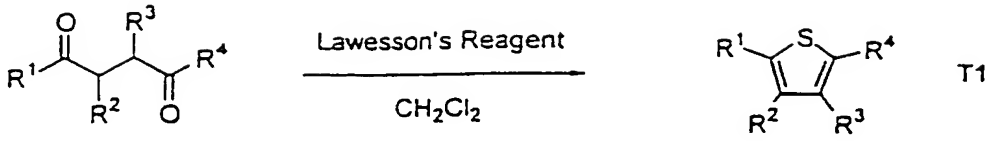
Can also be used to make:

alternative 1,4-dione synthesis, particularly:

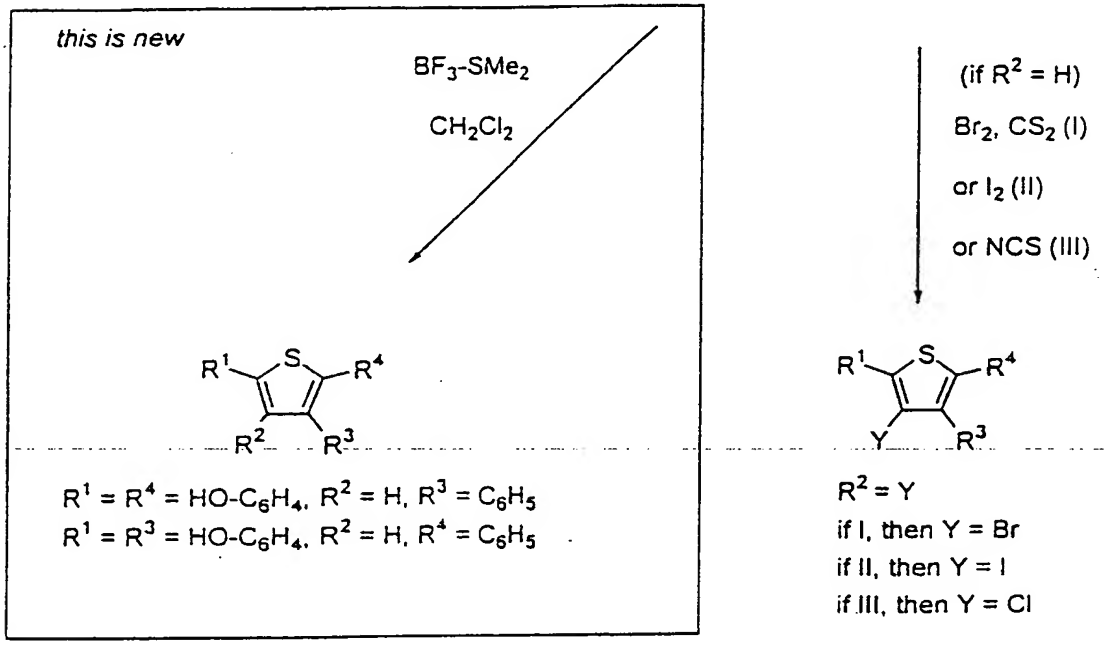


$R^1 = C_6H_5$, C_6H_4-OR, R = CH_3, H
$R^3 = C_6H_5$, C_6H_4-OR', R' = CH_3, H
$R^5 = C_6H_5$, C_6H_4-OR", R" = CH_3, H

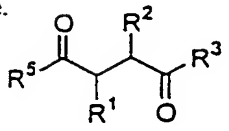
Scheme 9



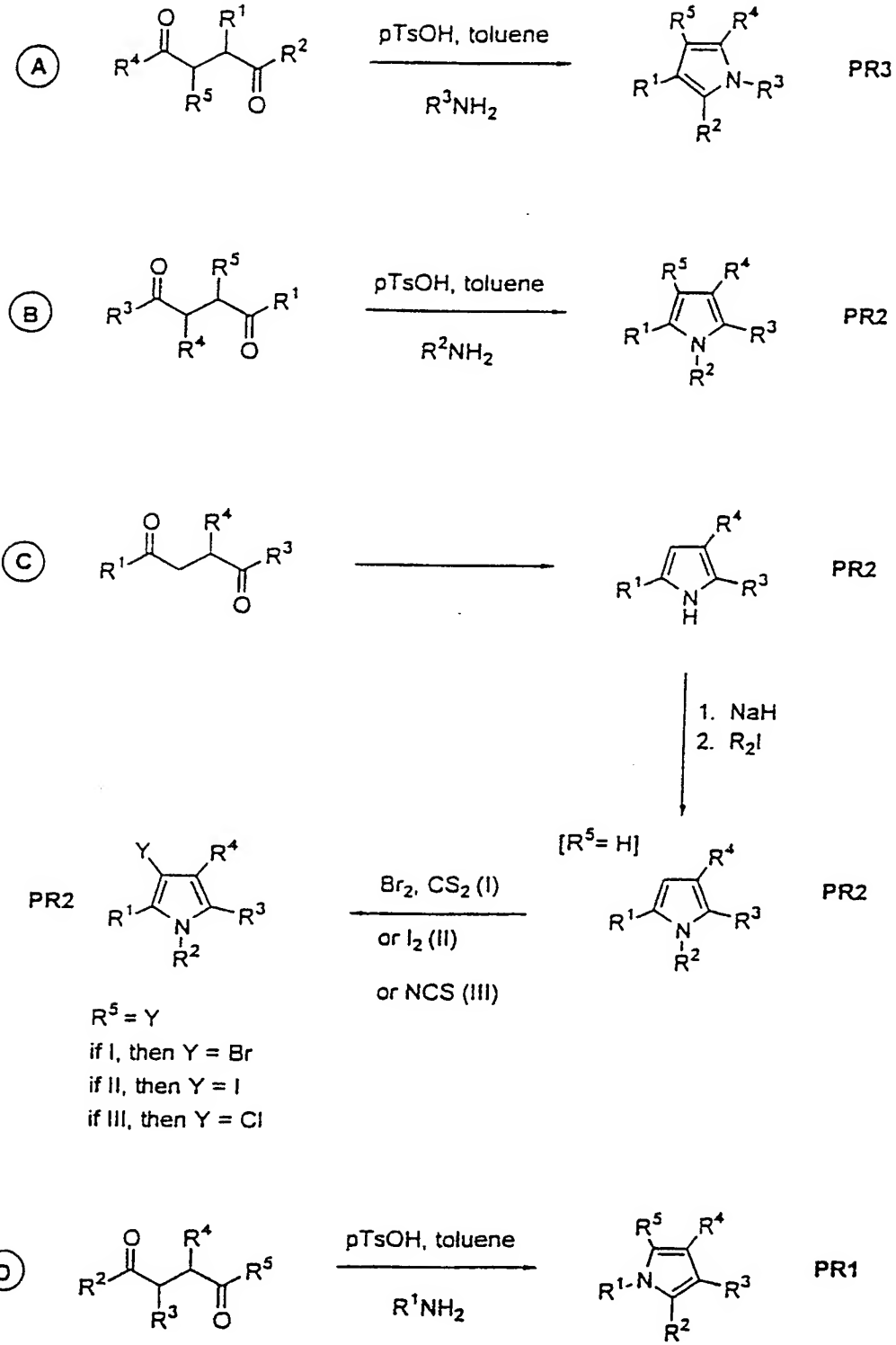
60 $R^1 = R^4 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^3 = C_6H_5$
61 $R^1 = R^3 = p\text{-}CH_3O\text{-}C_6H_4$, $R^2 = H$, $R^4 = C_6H_5$



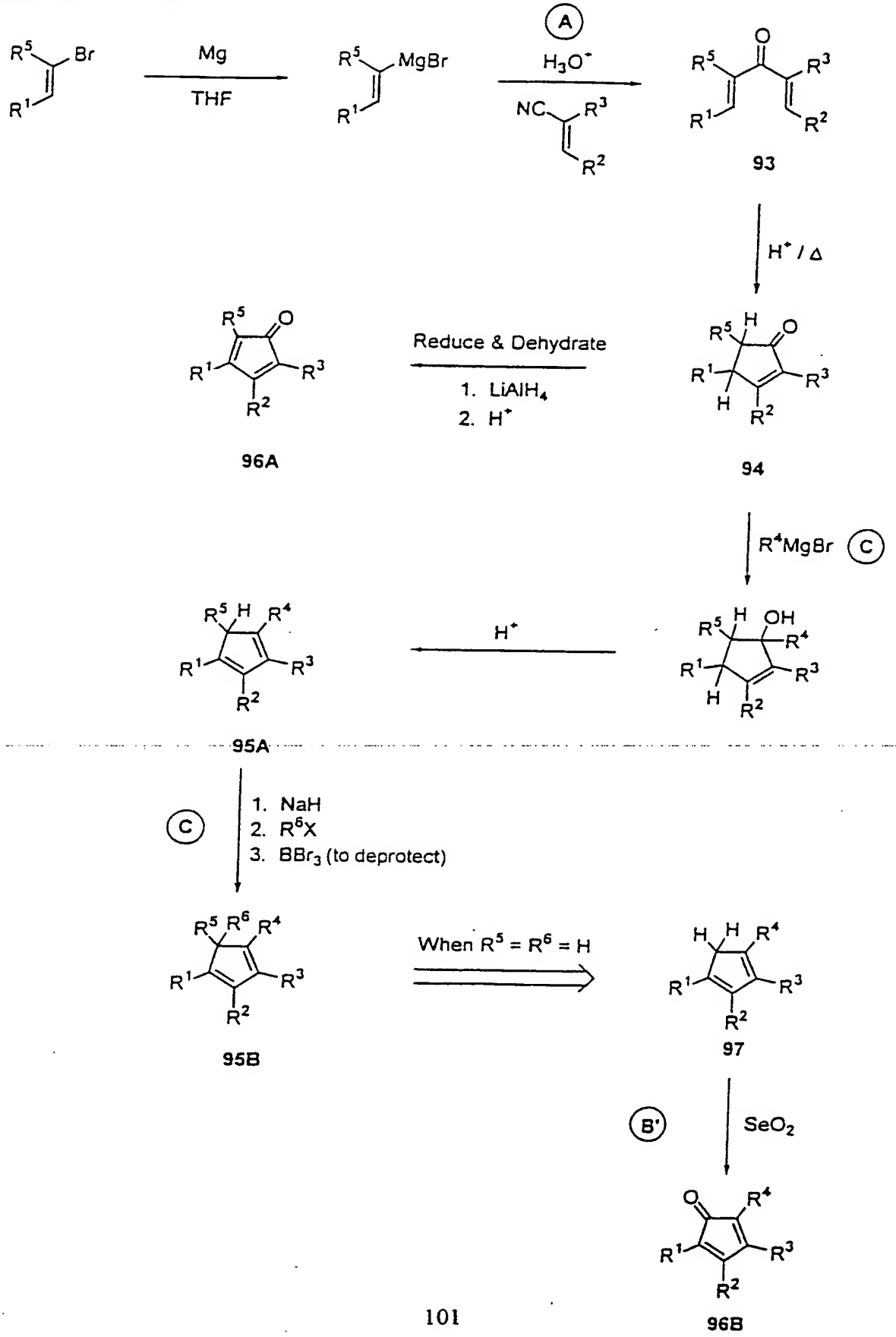
T2 Synthesized by choice of starting diketone i.e.



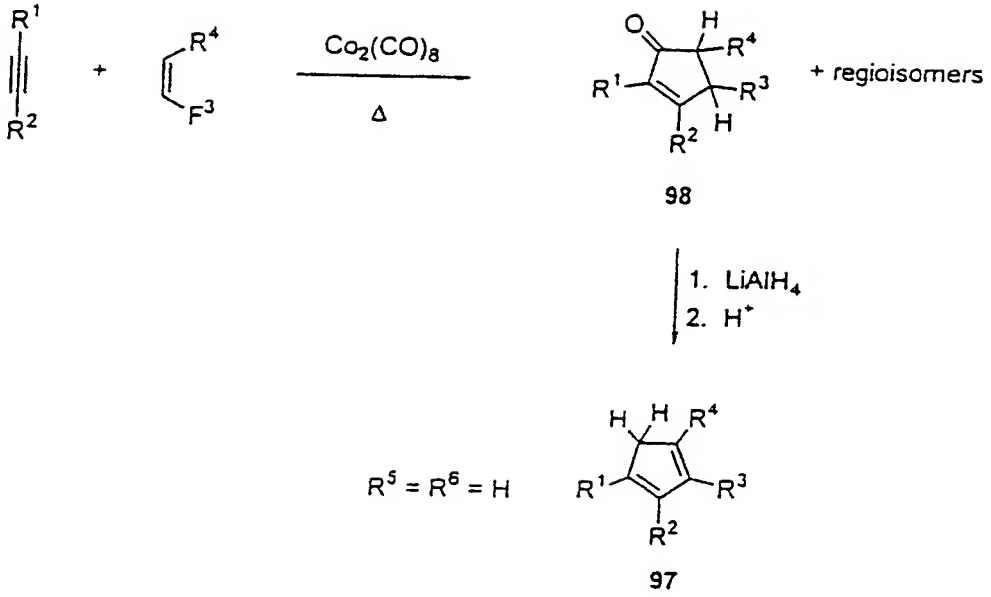
Schemes 10A-D



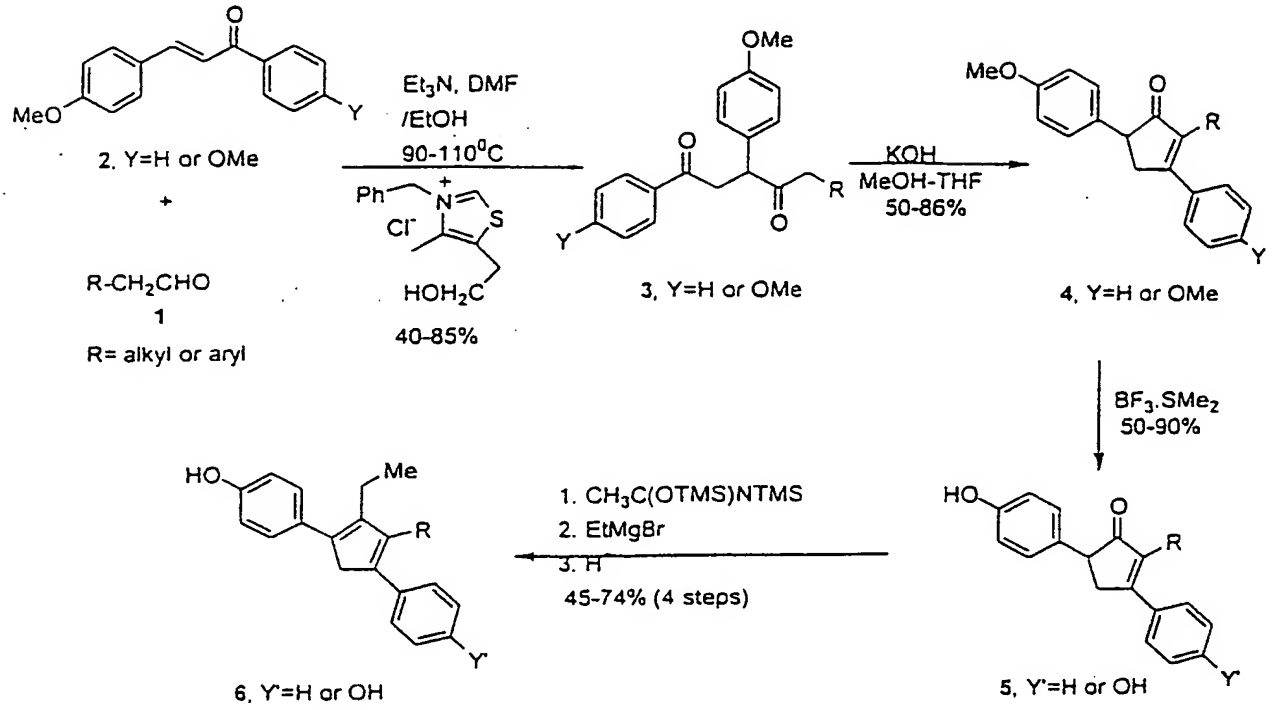
Scheme 11A



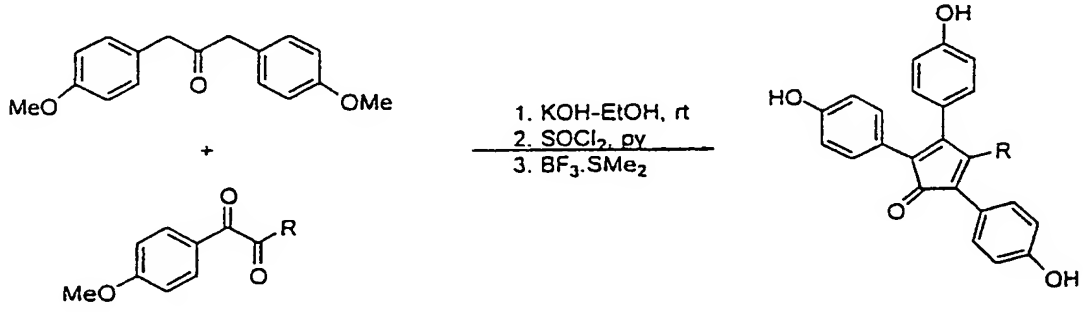
Scheme 11B



Scheme 11C: General Route for the Synthesis of Cyclopentadienes

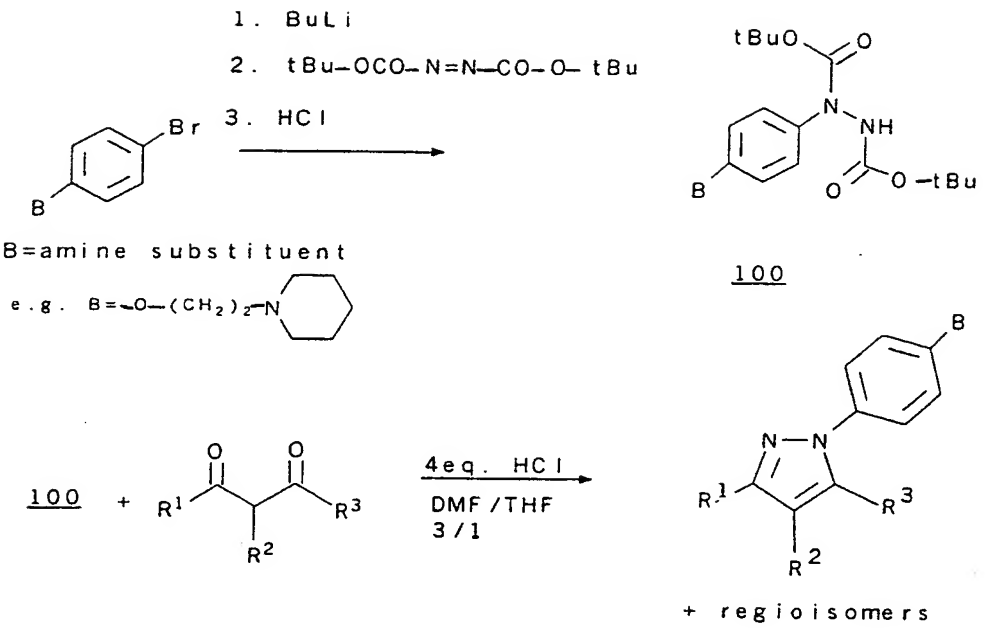


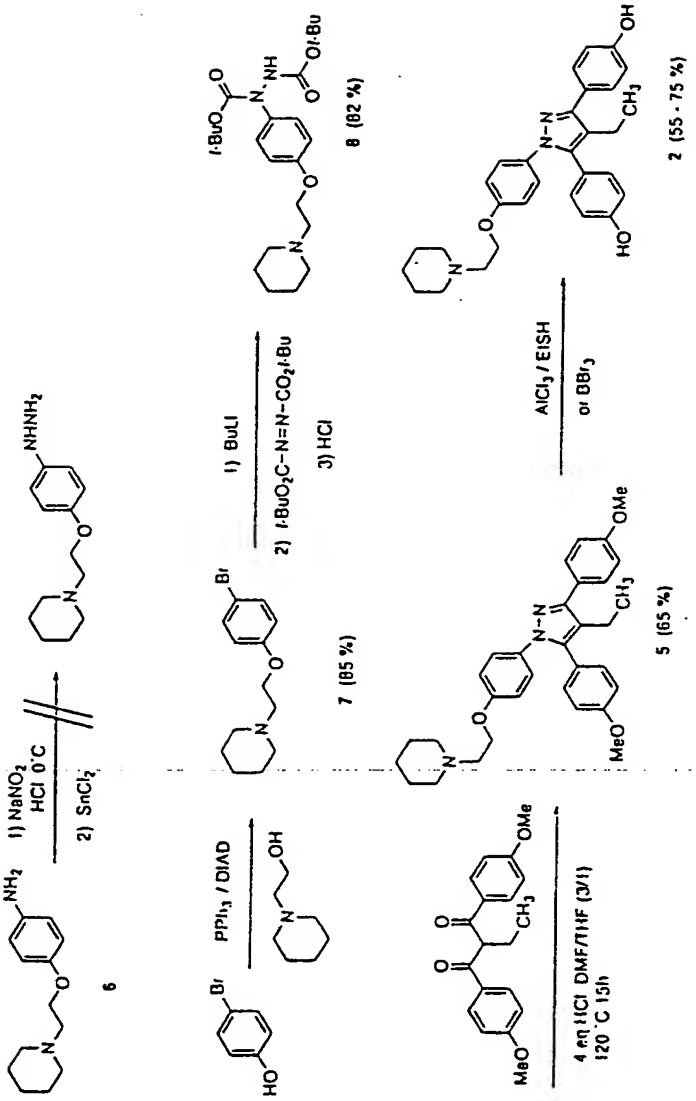
Scheme 11D: General Route for the Synthesis of Cyclopentadienones



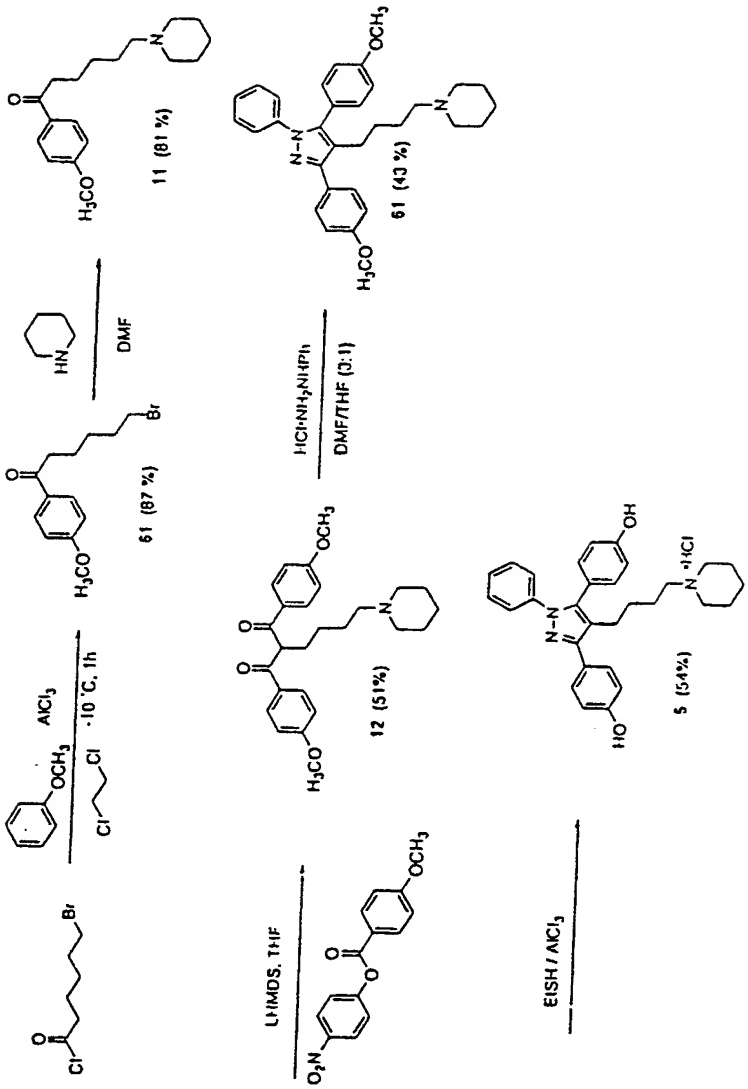
R = Et or *n*-Pr

Scheme 12A





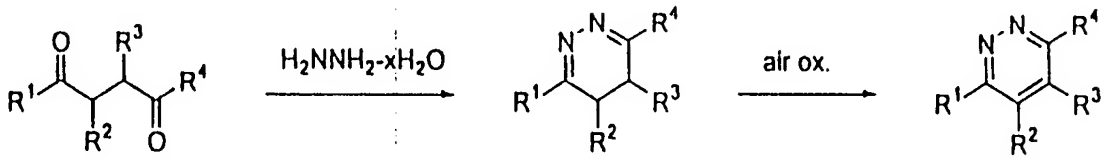
Scheme 12B. Synthesis of N(1) basic side chain containing pyrazole 2.



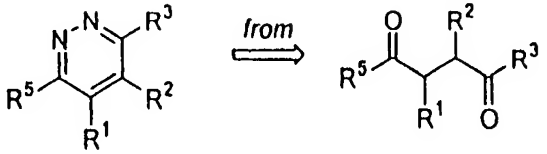
Scheme 12C. Synthesis of C(4) basic side chain containing pyrazole 3. 20

Scheme 13A

Pyridazine synthesis particularly: $R^1 = C_6H_5, C_6H_4\text{-}OR, R = CH_3, H$
$R^2 = C_6H_5, C_6H_4\text{-}OR', R' = CH_3, H$
$R^4 = C_6H_5, C_6H_4\text{-}OR'', R'' = CH_3, H$

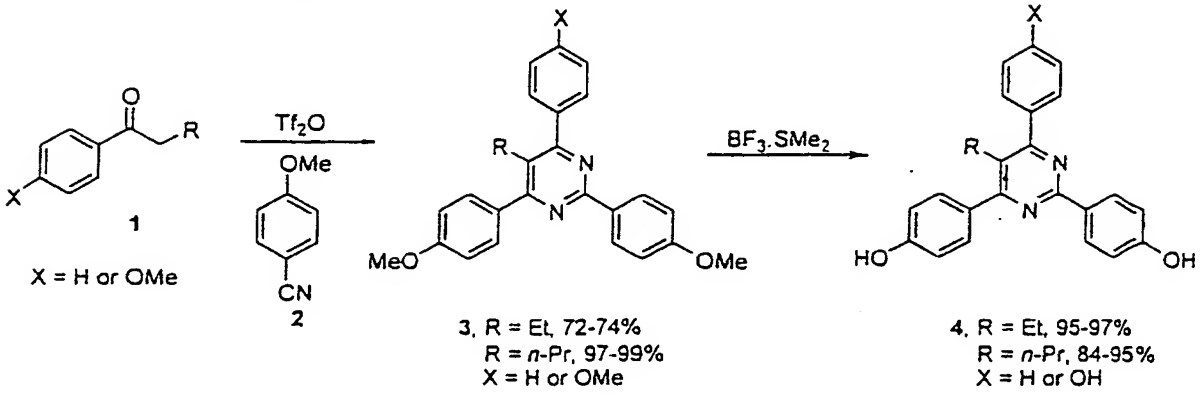


Can also be used to make:

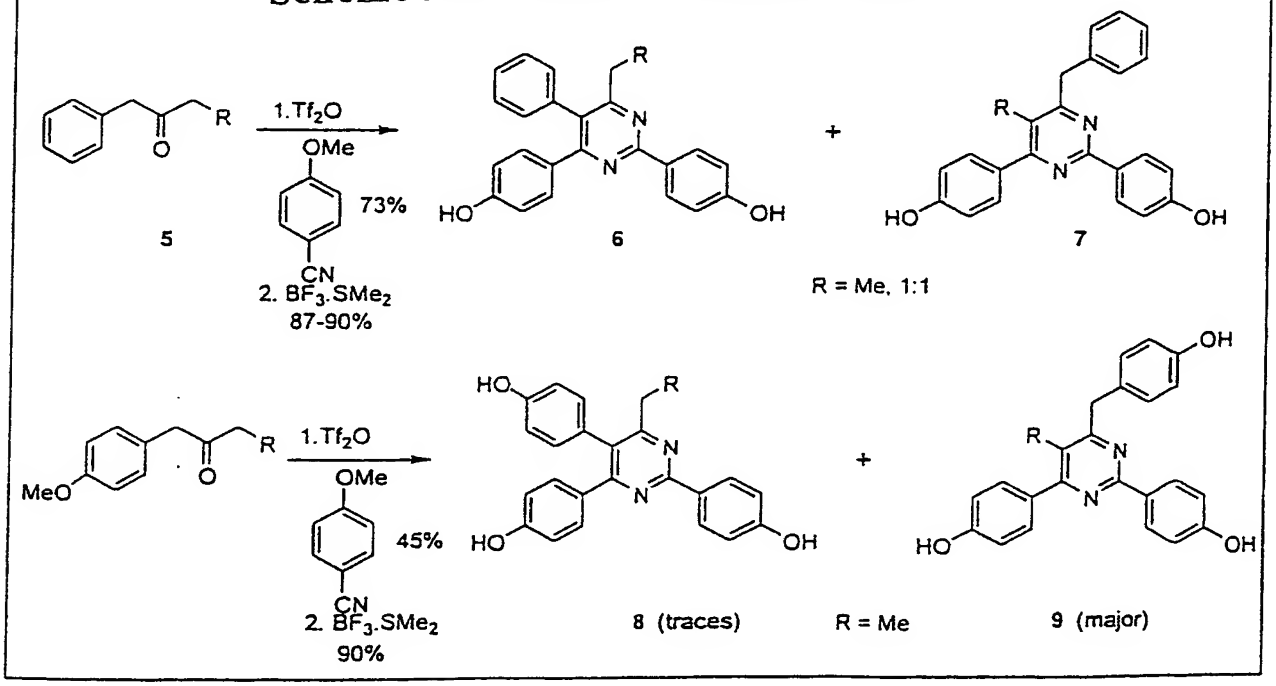


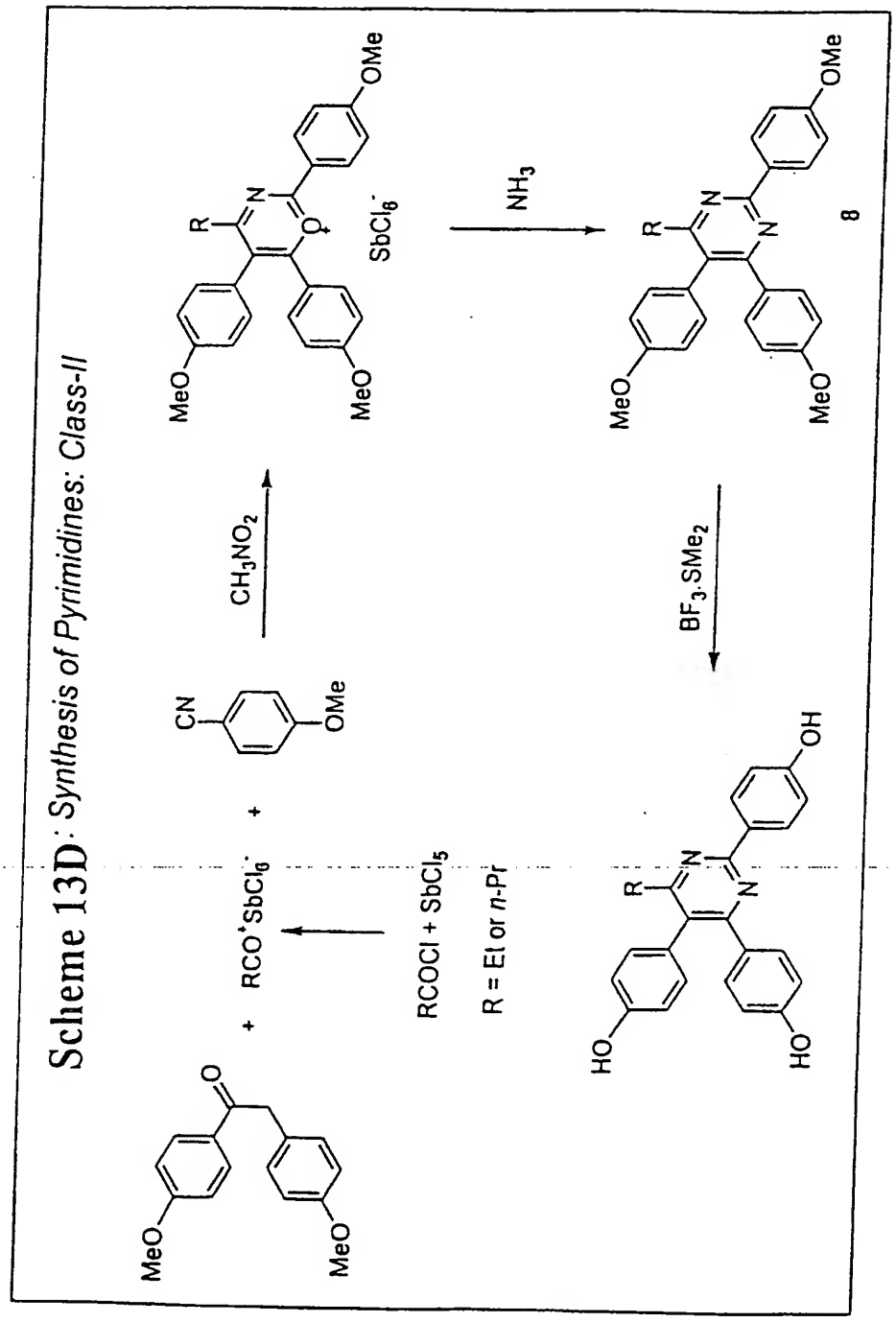
particularly: $R^1 = C_6H_5, C_6H_4\text{-}OR, R = CH_3, H$
$R^3 = C_6H_5, C_6H_4\text{-}OR', R' = CH_3, H$
$R^5 = C_6H_5, C_6H_4\text{-}OR'', R'' = CH_3, H$

Scheme 13B: *Synthesis of Pyrimidines: Class-I*

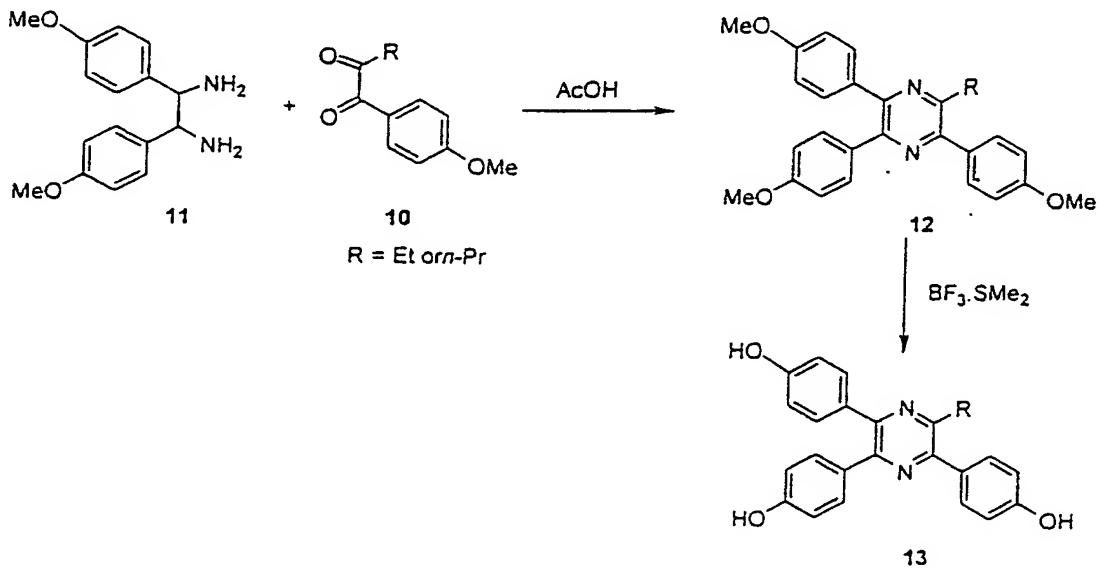


Scheme 13C: *Synthesis of Pyrimidines: Class-II*

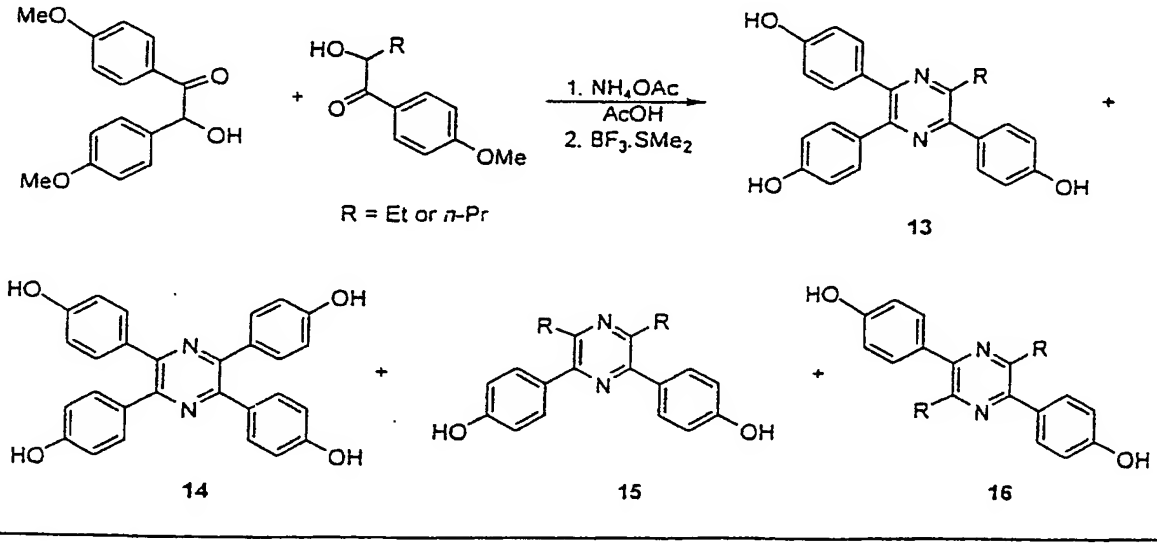




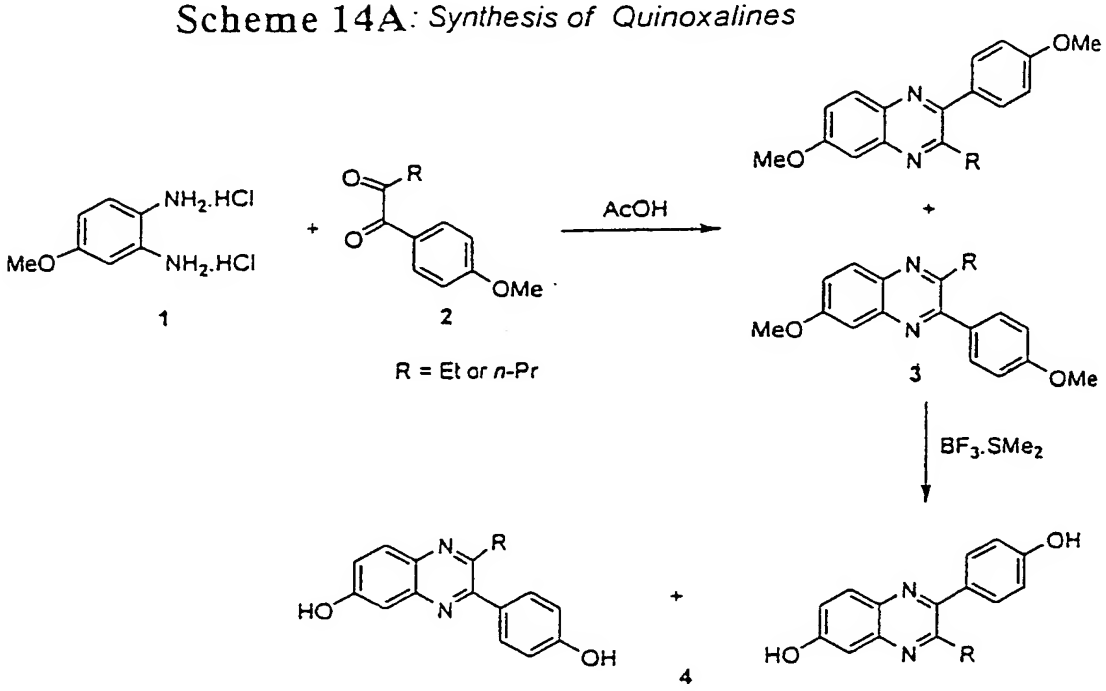
Scheme 13E: *Synthesis of Pyrazines*



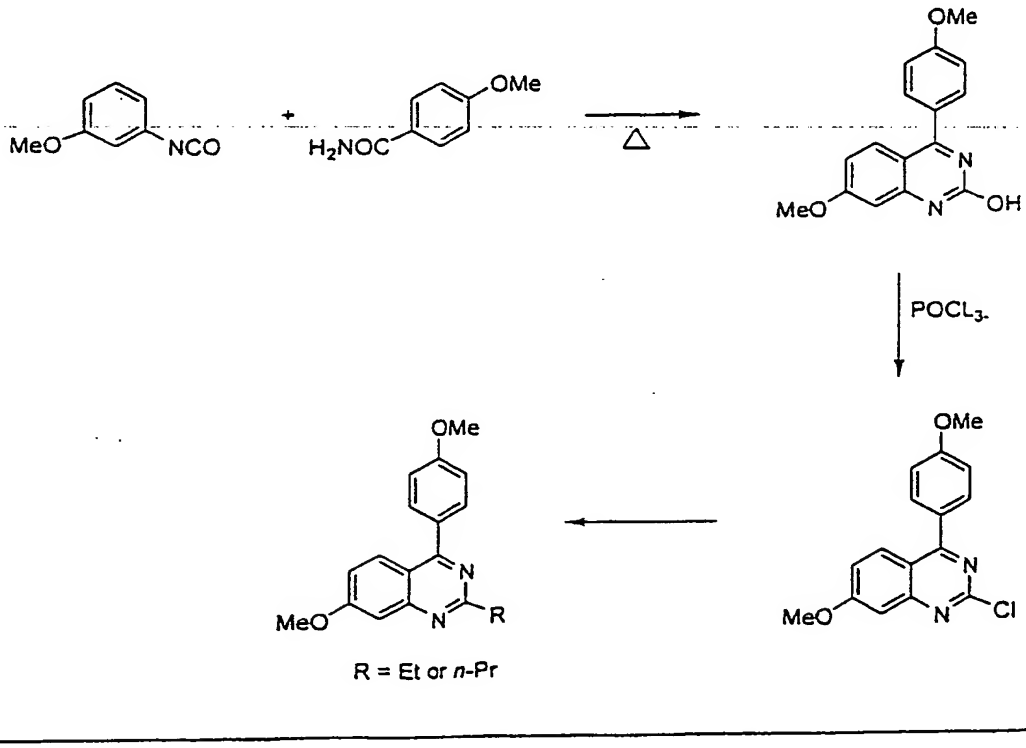
Scheme 13F: *Synthesis of Pyrazines*



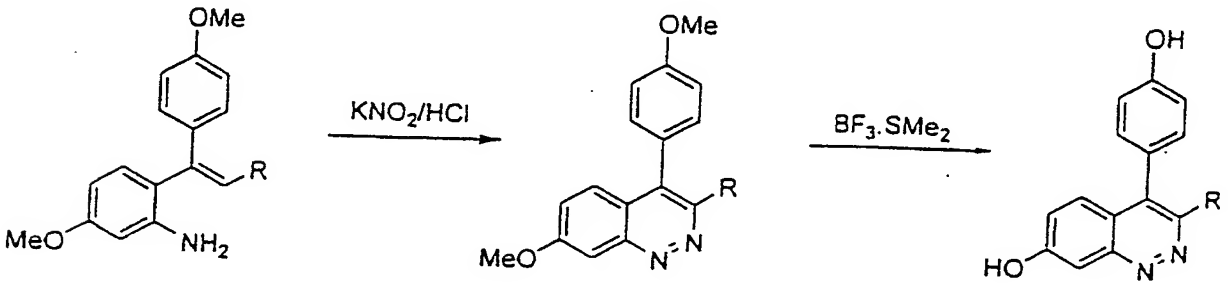
Scheme 14A: *Synthesis of Quinoxalines*



Scheme 14B: *Synthesis of Quinazolines*

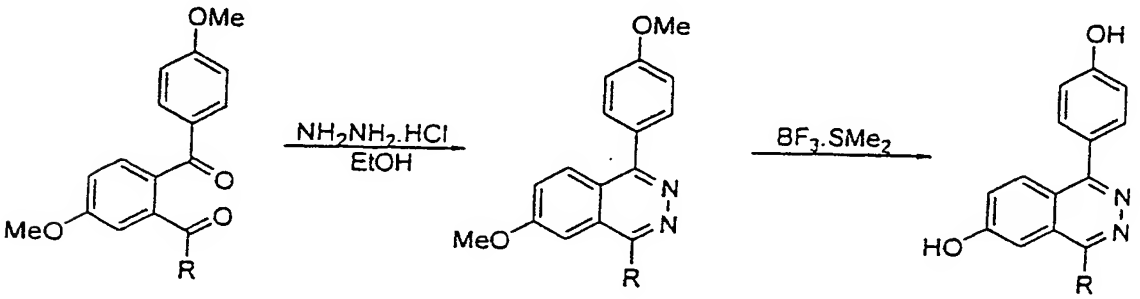


Scheme 14C: *Synthetic route to cinnolines*



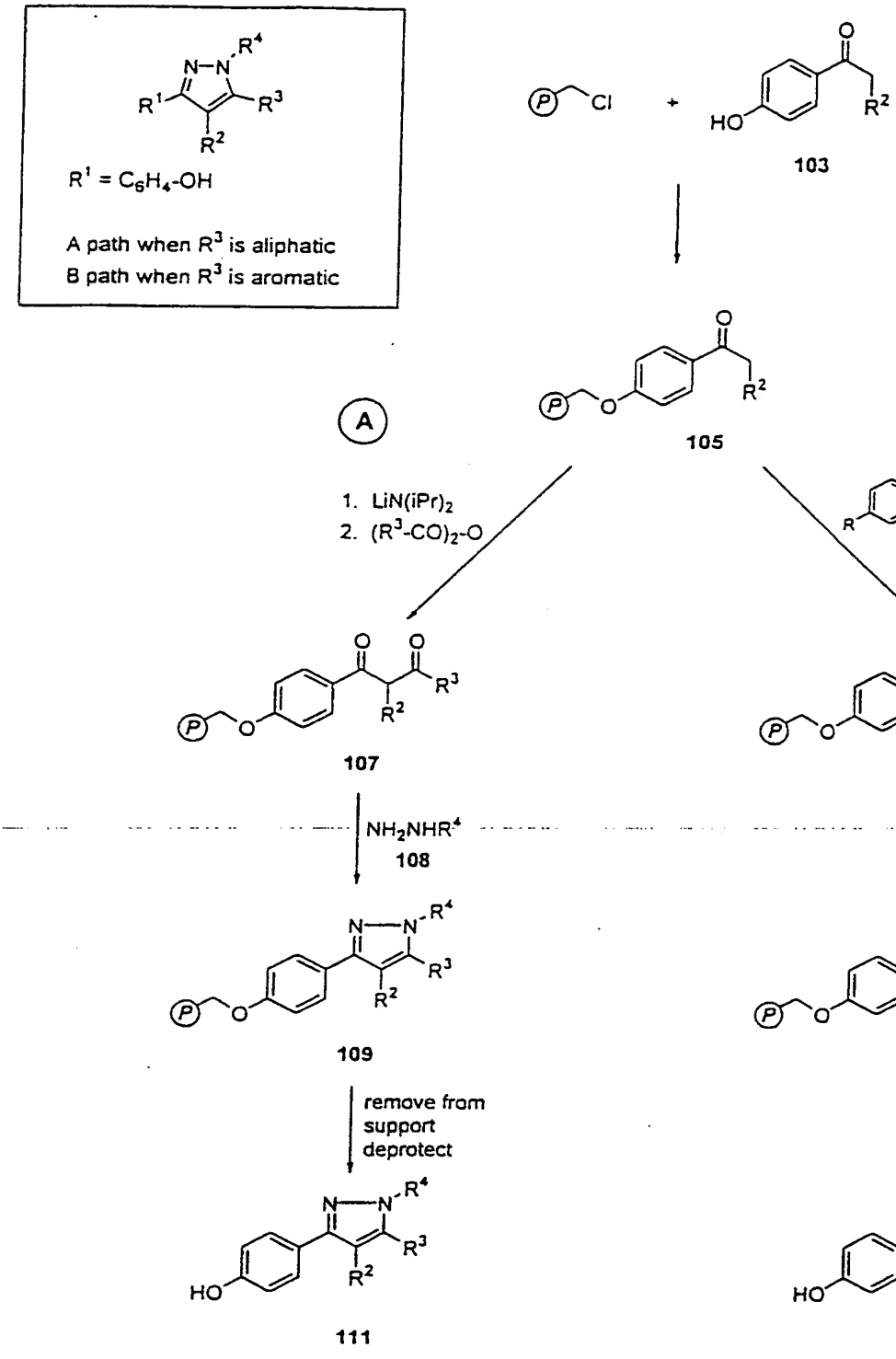
R = Et or *n*-Pr

Scheme 14D: *Synthetic route to phthalazines*

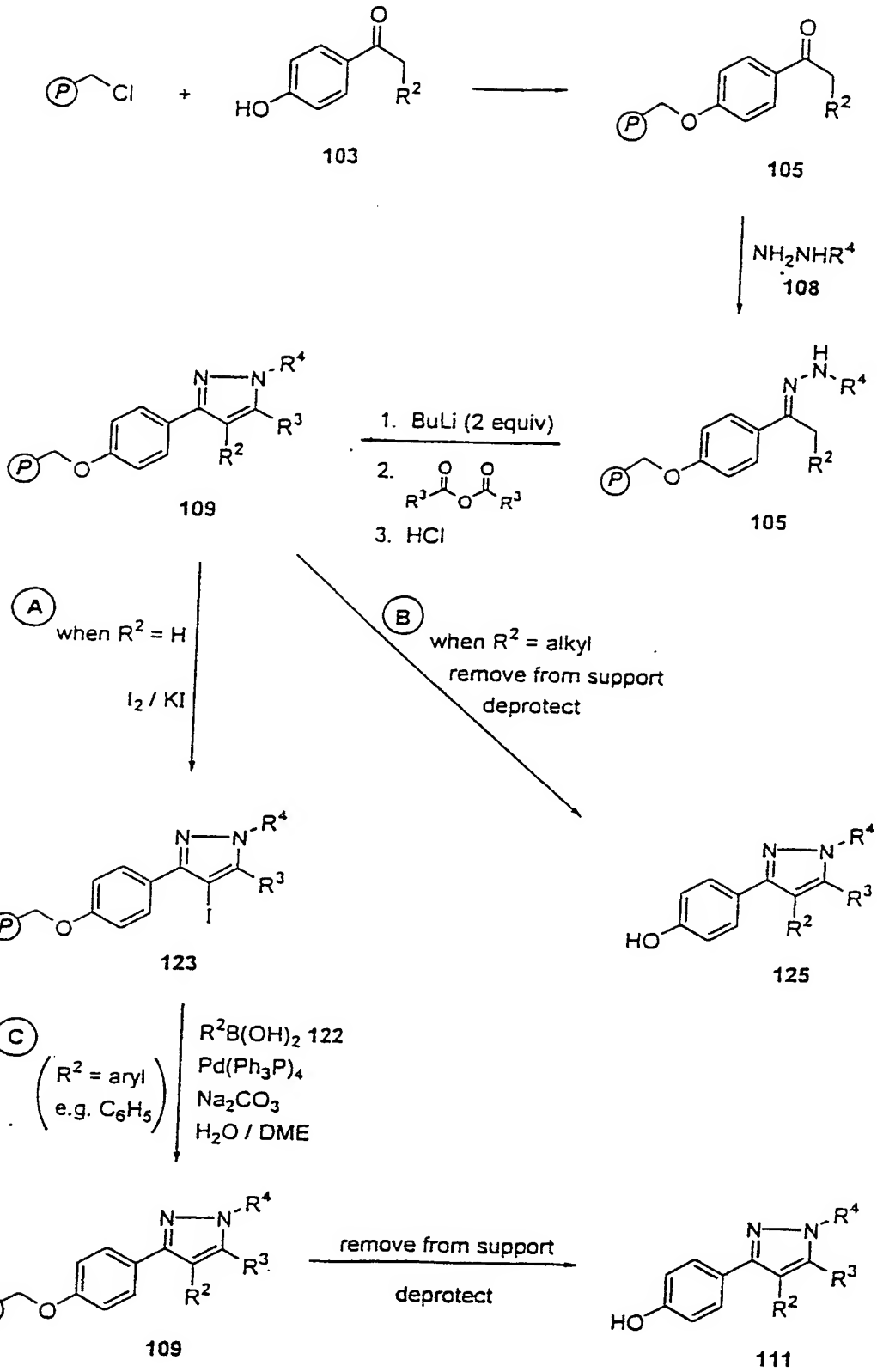


R = Et or *n*-Pr

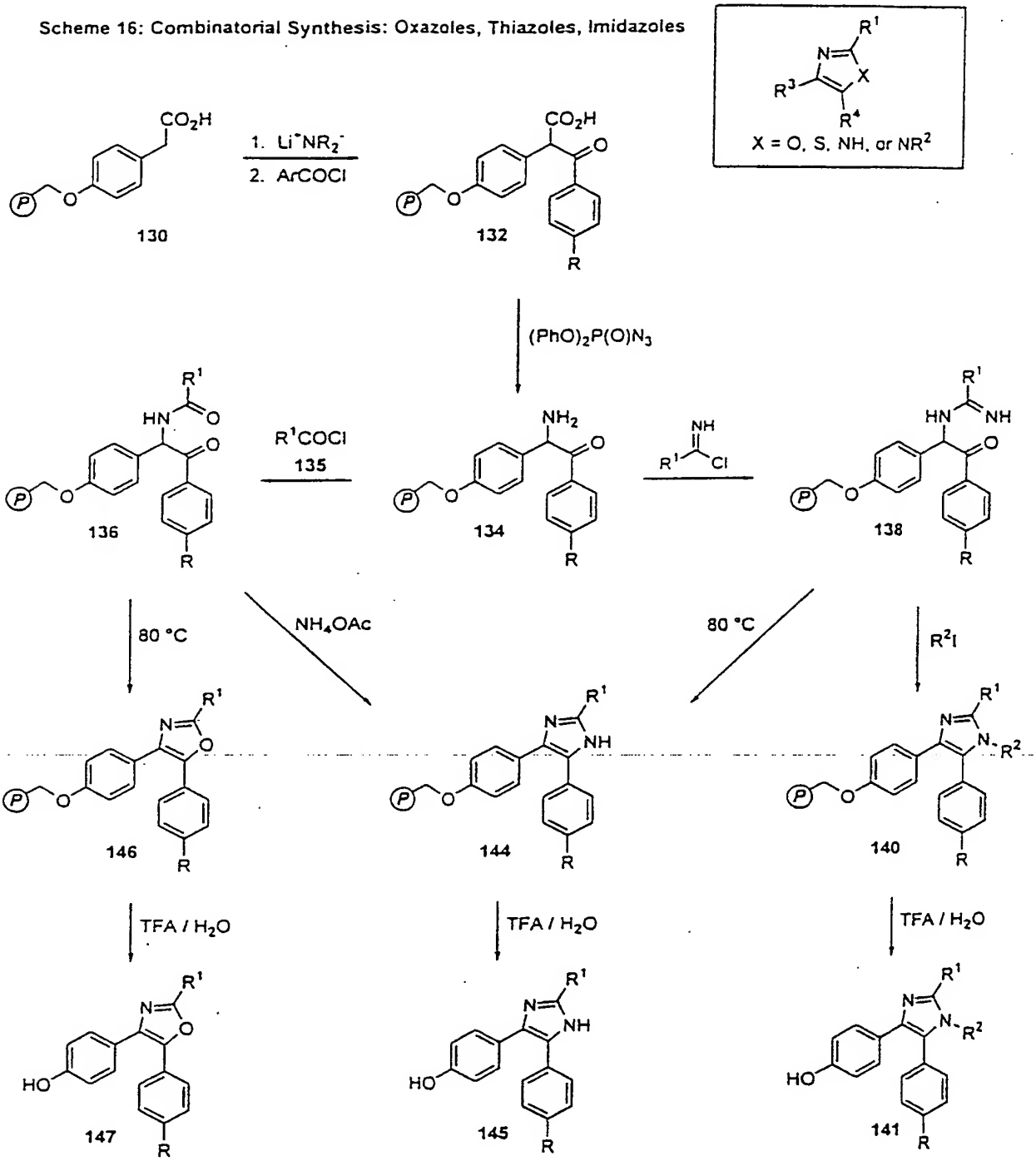
Scheme 15A: Combinatorial Synthesis: Pyrazoles



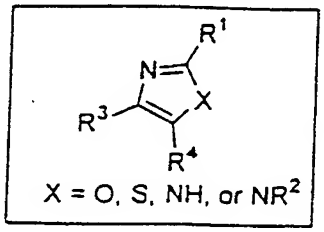
Scheme 15B: Combinatorial Synthesis: Pyrazoles(II)

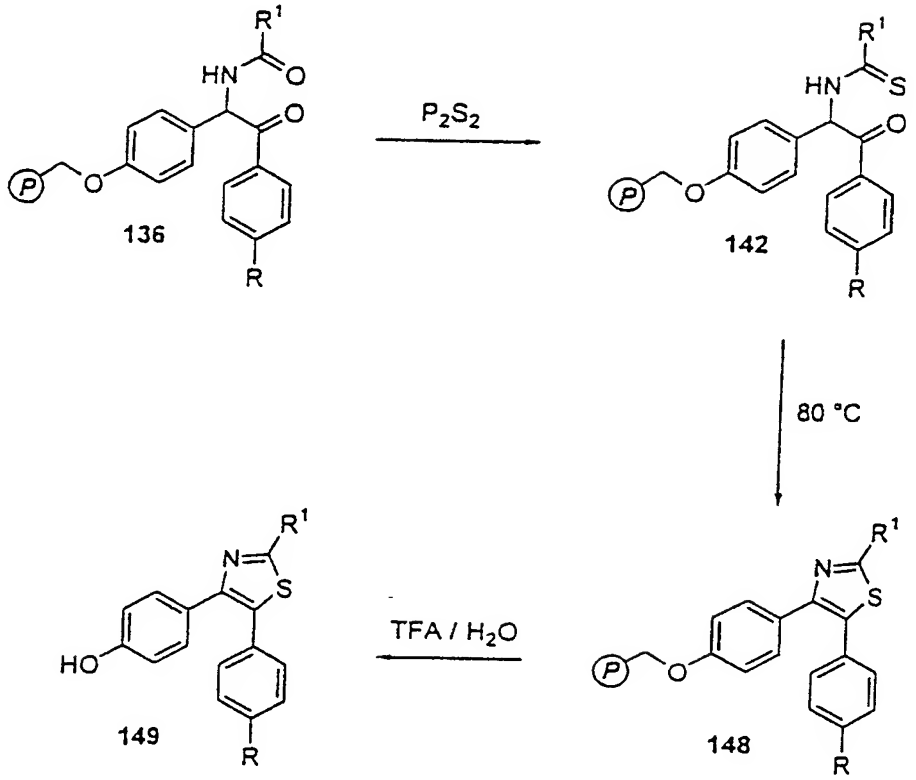


Scheme 16: Combinatorial Synthesis: Oxazoles, Thiazoles, Imidazoles

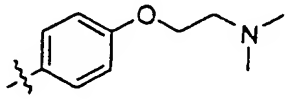


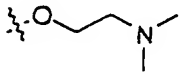
Scheme 16: Combinatorial Synthesis: Oxazoles, Thiazoles, Imidazoles (cont)

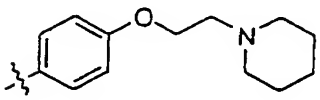


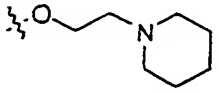


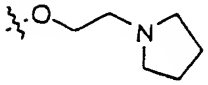
Scheme 17: Exemplary Basic and Polar Substituents

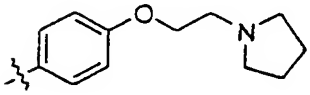


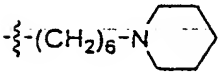


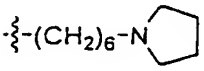


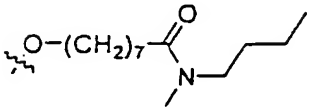


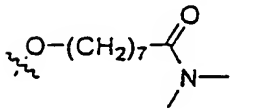


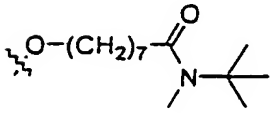


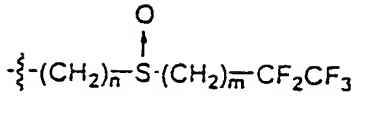


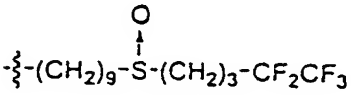


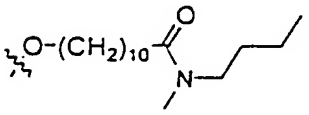


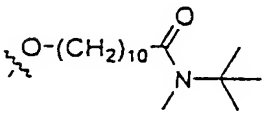


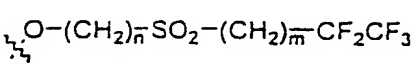


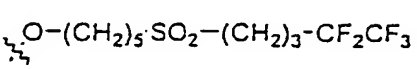




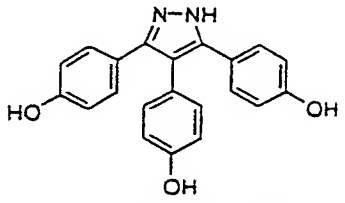




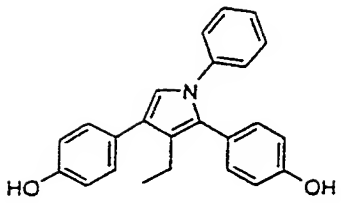


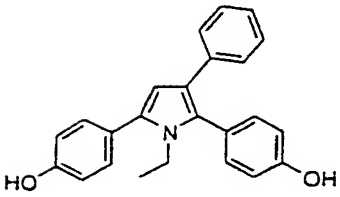


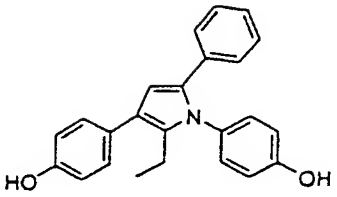
Scheme 18: Exemplary ER Ligands



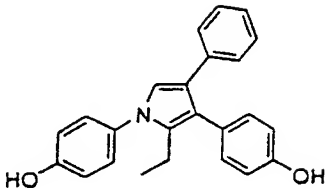
RBA = 0.016%

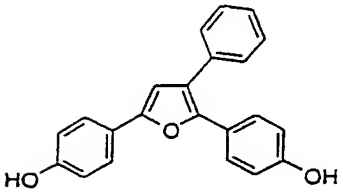




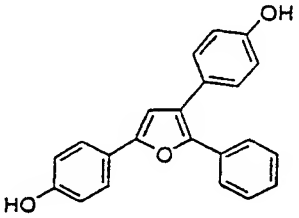


RBA = 0.50%

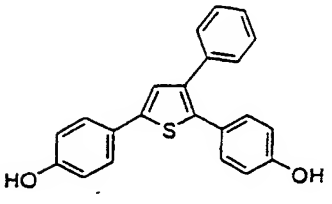




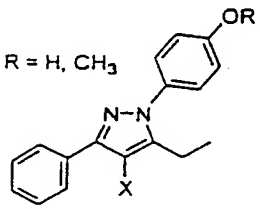
RBA = 0.05%

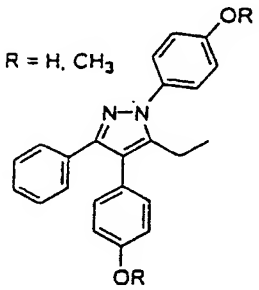


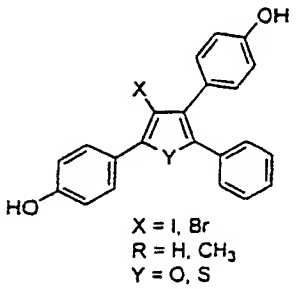
RBA = 0.135%



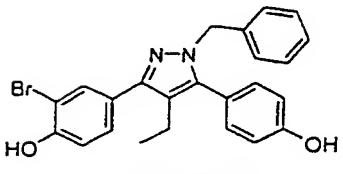
RBA = 0.03%



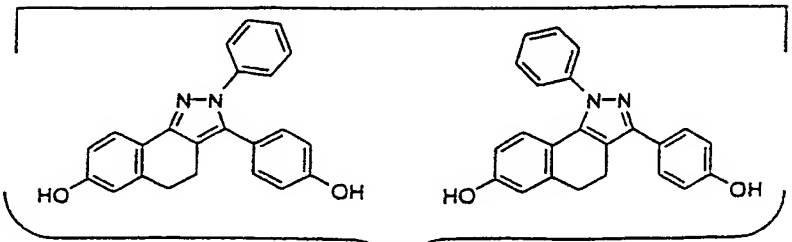




X = I, Br
R = H, CH_3
Y = O, S

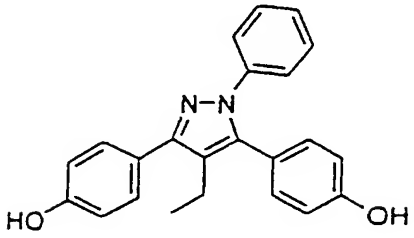


RBA = 0.12%

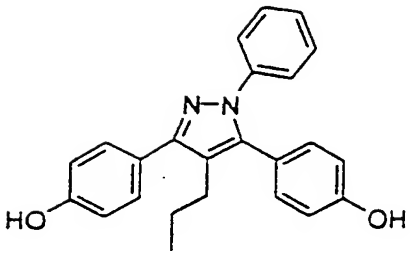


RBA = 0.68 % (1:1 mixture of isomers)

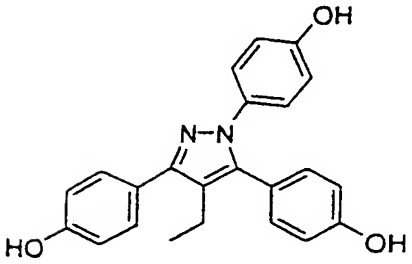
Scheme 18 (Continued)



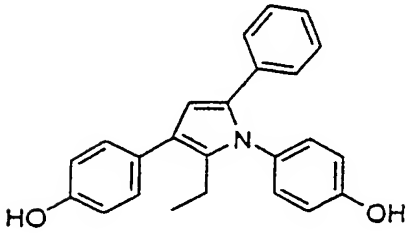
38b
RBA = 14%



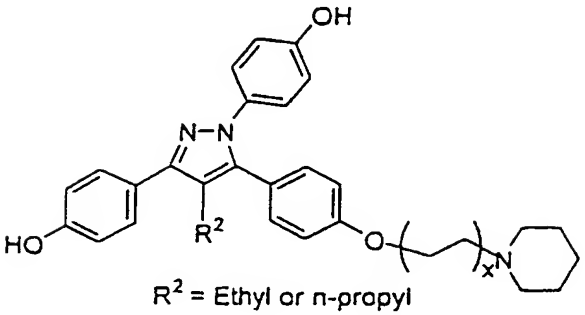
RBA = 25%



38d
RBA = 20%

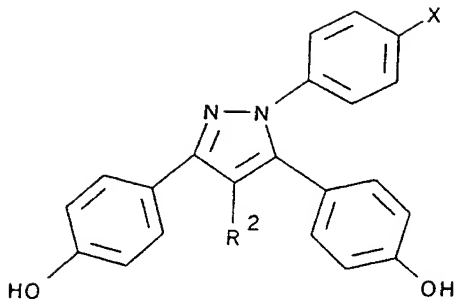


RBA = 0.50%



R^2 = Ethyl or n-propyl

Scheme 18 (continued)



334 R^2= n-propyl
X=H
RBA=25
ER_α=53
ER_β=0.54

335 R^2=ethyl
X=OH
RBA=19
ER_α=31.6
ER_β=0.30

336 R^2= n-propyl
X=OH
RBA=30
ER_α=63
ER_β=0.095

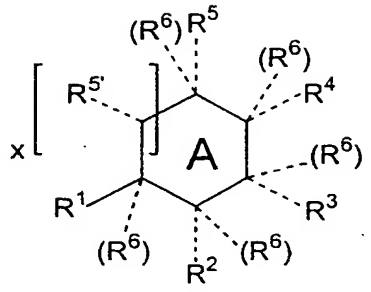
333 R^2=ethyl
X=H
RBA=14
ER_α=60±16
ER_β=18±4

339 R^2=i Butyl
X=OH
RBA=23

339d R^2=i Butyl
X=H
RBA=4.3

We claim:

1. An estrogen receptor ligand having the structure:



wherein x is 0 or 1 and when x = 0 the core ring A is a 5 -membered ring structure that is doubly unsaturated or when x is 1 the core ring is a 6-membered ring structure which is aromatic wherein the ring can be a carbocyclic ring or a heterocyclic ring having one or two non-carbon heteroatom and wherein:

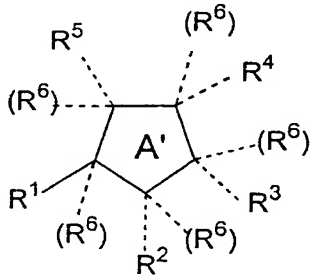
R^1 can be selected from the group consisting of phenyls and substituted phenyls wherein the non-hydrogen phenyl group substituents can include, without limitation, basic or polar groups, halogens, hydroxy groups, lower alkyl, alkenyl, alkynyl, and alkoxyl groups, lower ethers, ketones, or thioethers, and substituted lower alkyl, alkenyl or alkynyl groups, where the substituents can be halogens or hydroxy groups;

R^2, R^3 and R^4, can be the same or different, and can be selected from the group consisting of hydrogen, a basic or polar group, a phenyl or substituted phenyl group, lower alkyl, alkenyl or alkynyl where the lower alkyl, alkenyl or alkynyl groups may be substituted, with a basic or polar group, a phenyl, hydroxyls or halogens, lower ethers, ketones or thioethers, and halogens;

R^5 or $R^{5'}$, when present, can be selected from any of the groups defined for R^2, R^3 and R^4 or may be hydrogens, R^5 and $R^{5'}$ may be the same as or different than any of R^1, R^2, R^3, or R^4 and R^5 or $R^{5'}$ may be the same or different than each other; and

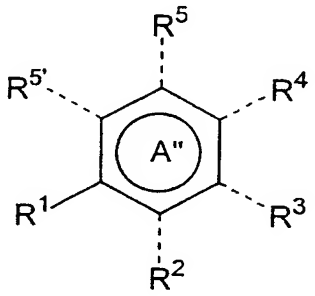
R^6, when present, can be hydrogen, a basic or polar group, a lower alkyl, alkenyl, alkynyl, or alkoxy groups, which may be substituted with lower alkyl, alkenyl or alkynyl groups, lower ether or thioethers or halogens and one or more of any $-CH_2-$ groups in R^6 can be replaced with -CO- groups.

2. The estrogen receptor ligand of claim 1 which has the structure:



wherein A' is a 5 -membered ring structure that is doubly unsaturated.

3. The estrogen receptor ligand of claim 1 which has the structure:



wherein A" is a 6-membered ring structure which is aromatic.

4. The estrogen receptor ligand of claim 1 that is a pyrazole.

5. The estrogen receptor ligand of claim 1 that is a cyclopentadiene.

6. The estrogen receptor ligand of claim 1 that is a furan.

7. The estrogen receptor ligand of claim 1 that is a pyrimidine.

8. The estrogen receptor ligand of claim 1 wherein R^1 is a p-OH-phenyl group.

9. The estrogen receptor ligand of claim 8 wherein R^3 is a p-OH-phenyl group.

10. The estrogen receptor ligand of claim 8 wherein R^2 is a lower alkyl group.

11. The estrogen receptor ligand of claim 8 wherein R3 is a phenyl group substituted with a basic or polar group.

12. The estrogen receptor ligand of claim 1 wherein R^2 is an ethyl or an i-propyl group.

13. A pharmaceutical composition comprising an estrogen receptor of claim 1 in an amount sufficient to exhibit an effect on a hormone-dependent disorder.

14. A method for treating a hormone-dependent disorder which comprises the step of administering to a patient suffering from that disorder the pharmaceutical composition of claim 13.

15. The method of claim 14 wherein the hormone-dependent disorder is hormone-responsive breast cancer.

16. A method for treating estrogen responsive disorders and physiological conditions which comprises the step of administering to a patient suffering from the disorder a pharmaceutical composition of claim 14.

17. A method for selective regulation of a cellular activity under the control of estrogen receptor which comprises administering a composition comprising an amount of an estrogen ligand of claim 1 sufficient to effect such cellular activity.

18. The estrogen receptor ligand of claim 1 which exhibits RBA of about1% or more.

19. The estrogen receptor ligand of claim 1 which exhibits RBA of about 10% or more.

20. The estrogen receptor ligand of claim 1 which exhibits selective affinity for one of ERα or ERβ.

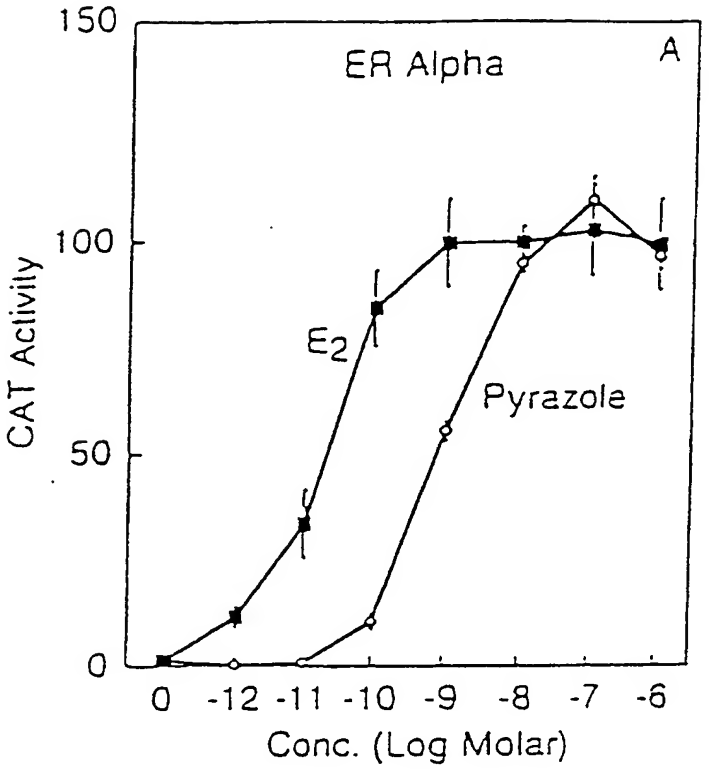


FIG. 1A

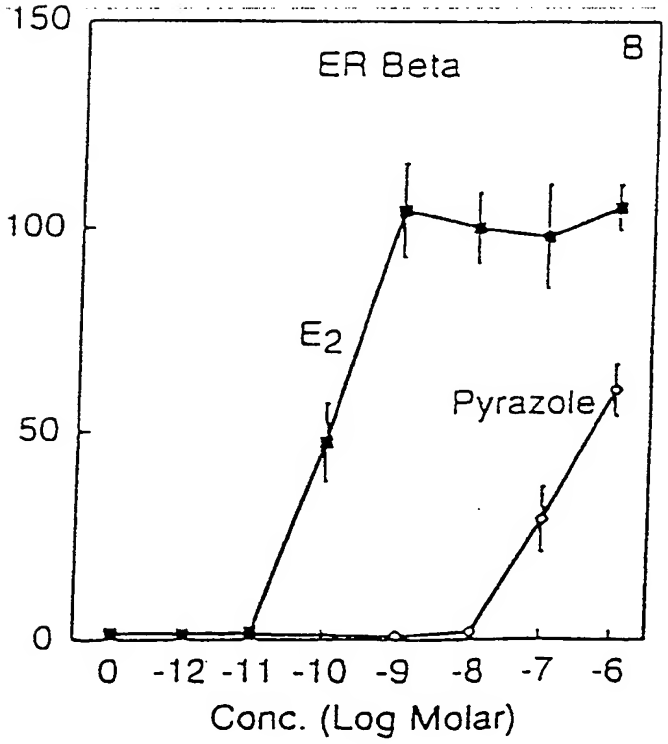


FIG. 1B

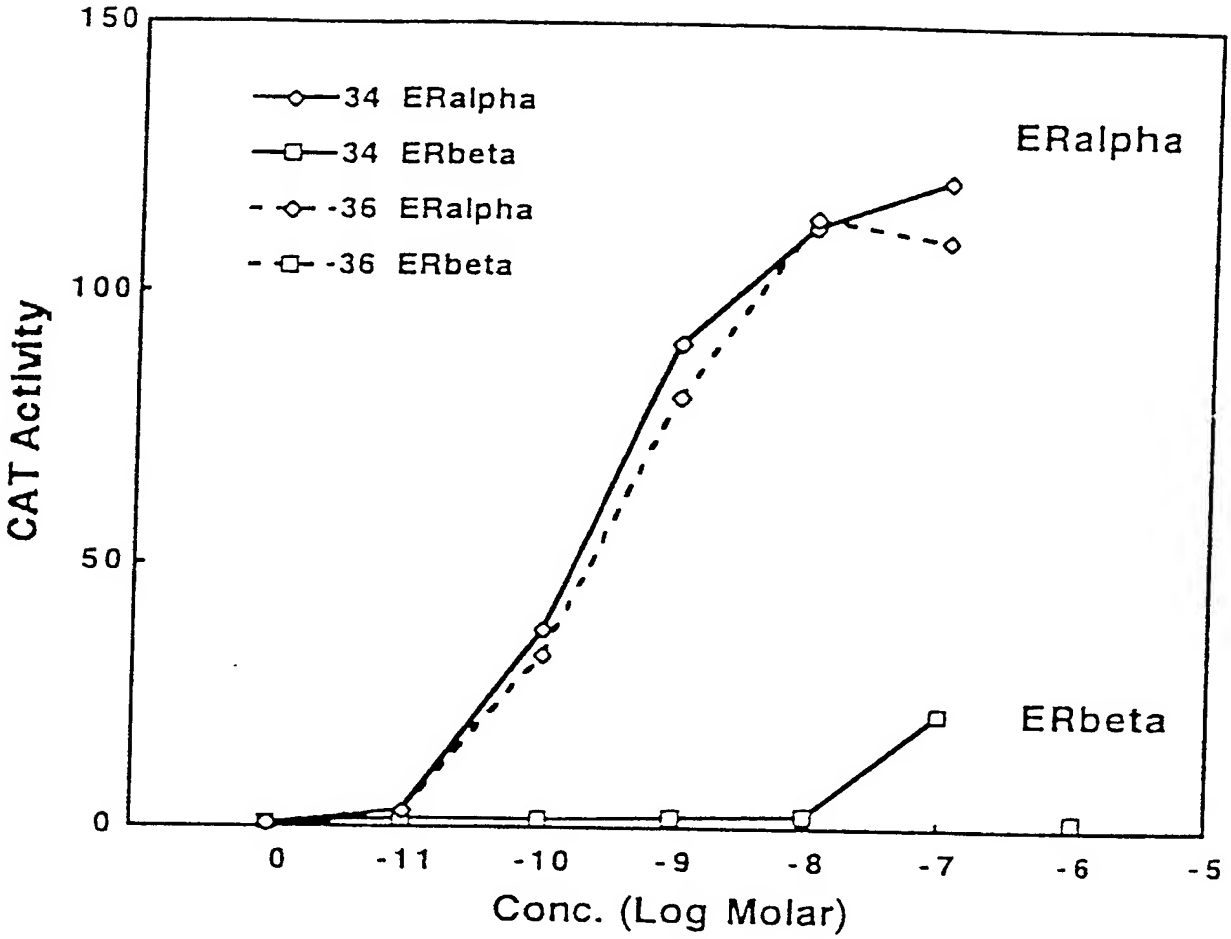


FIG. 2

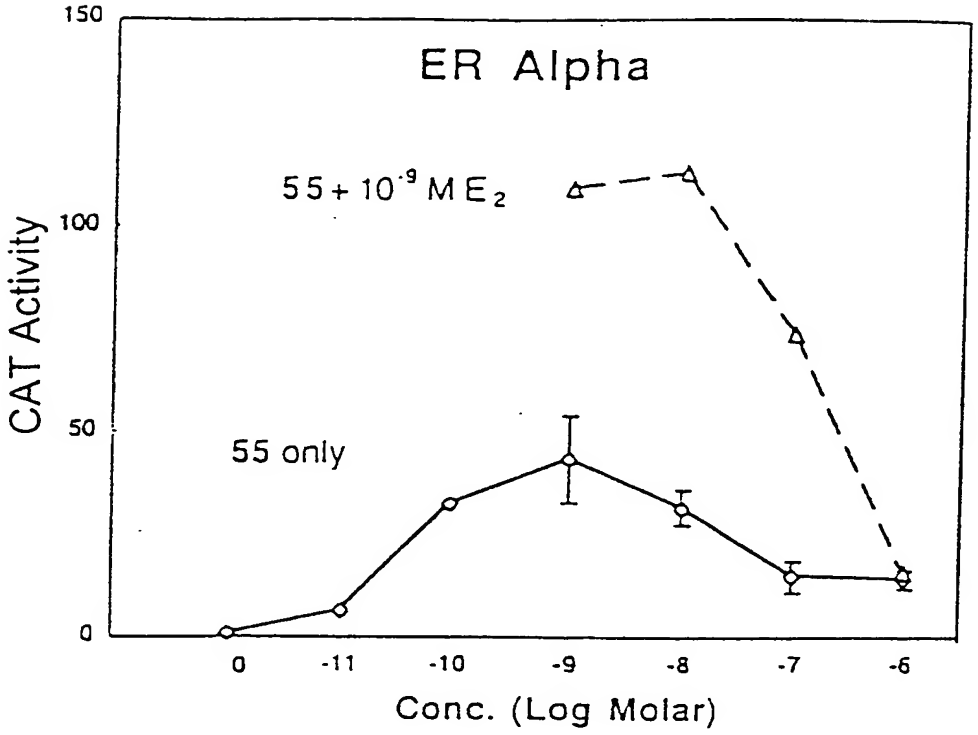


FIG. 3A

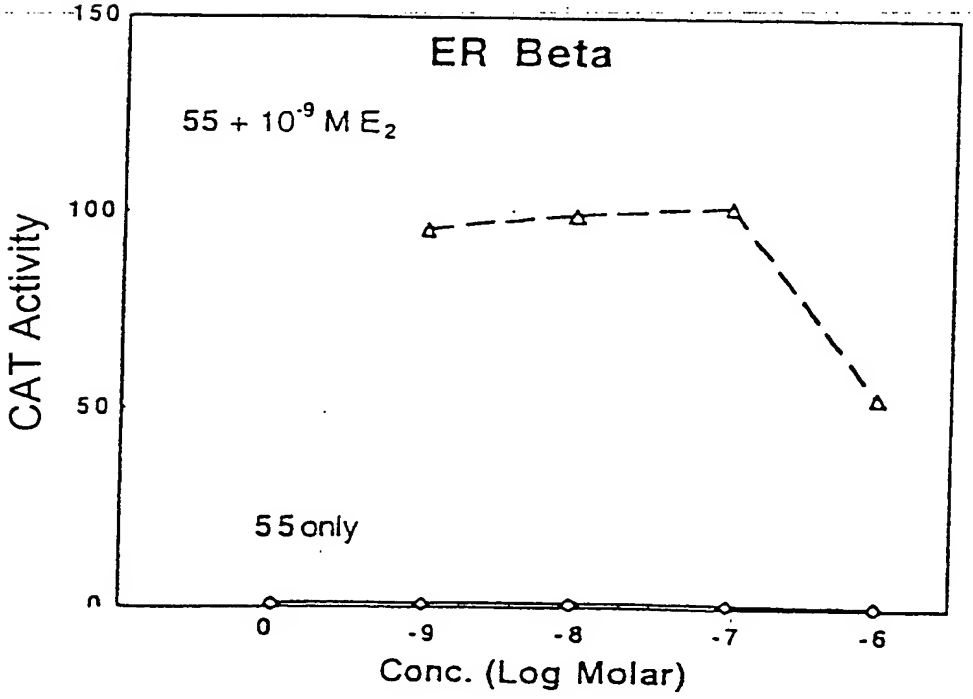


FIG. 3B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/22747

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/05, 31/341, 31/415, 31/505; C07C 39/12, 39/17; C07D 231/12, 237/08, 307/36
US CL : 514/256, 406, 461, 729, 736; 544/242; 548/377.1; 549/506; 568/731, 744

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/256, 406, 461, 729, 736; 544/242; 548/377.1; 549/506; 568/731, 744

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN/CAS, structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,629,340 A (KUWANO et al.) 13 May 1997 (13.05.97), see entire docucment.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 February 2000 (04.02.2000)	24 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Richard L. Raymond Telephone No. (703) 308-1235

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